

MYCORRHIZA AND SOIL FERTILITY ATTRIBUTES FOR  
IMPROVED TRIPARTITE SYMBIOSIS OF  
PSOPHOCARPUS TETRAGONOLOBUS (L)  
DC ON A TYPIC EUTRUSTOX

by

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of Oklahoma State University  
in partial fulfillment of the requirements-  
for the degree of  
DOCTOR OF PHILOSOPHY  
December, 1985

Thesis  
1985D  
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## ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to Dr. J. Q. Lynd for his guidance, assistance, encouragement, and friendship during my graduate studies at Oklahoma State University.

To Drs. L. W. Reed, R. J. Crabtree, R. M. Ahring, and R. W. McNew, I express gratitude for serving on my committee. Special thanks are extended to Mrs. Fairy Lynd for friendship and everything during my work in the O.S.U. Soil Microbiology Laboratory.

My gratitude is expressed to my father, Mr. Vin Lurlarp, and my mother, Bunpuk Lurlarp, for being my wonderful parents.

Finally, very special thanks are expressed to the International Council for Development of Underutilized Plants (ICDUP) for their financial aid.

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## CHAPTER I

### INTRODUCTION

Legumes contribute excellent sources of protein and lipid for animal and human nutrition throughout the world. Proteins have long been recognized as essential for all living cell structure and metabolic functions including growth and reproduction of all organisms. Protein deficiency in human diets is of major importance within many developing countries of humid tropic regions.

The Winged Bean (Psophocarpus tetragonolobus (L) DC ) has great potential for alleviation of protein malnutrition throughout the neotropical countries, Asia, and Africa. The whole plant may be utilized as an edible foodstuff with high nutritive value. The young pods are sliced and prepared similar to French green beans. The young leaves are used as a leafy vegetable. Soup can be prepared with the unripe seeds. The ripe seeds may be roasted like peanuts. Tubers are prepared and eaten like potatoes. In addition, the plant is heavily nodulated with high nitrogen fixation capabilities well suited for soil improvement as a green manure, cover, and fallow restorative crop ( May, 1977; National Academy of Science, 1975; Pospisil et al., 1971; Thompson and Haryono, 1980 ). The green pods, leaves, seeds, and tuberous roots are high in protein and vitamin C ( Cerny et al., 1971; National Academy of Science, 1975; Pospisil et al., 1971 ). In Thailand and other countries of



Southeast Asia, pods are mainly eaten as a raw and cooked vegetable.

Winged bean plants grow from sea level up to 2,000 m, in humid conditions with short day length on Ultisol and Oxisol soil orders of humid tropical regions of the world ( National Academy of Science, 1975 ). The characteristics of these soils are highly weathered, acidic, clayey, leached under high rainfall condition. The composition of organic matter governs soil productivity with the continuous microbiological immobilization-mineralization cycling of available plant nutrients ( Lynd et al., 1983 ).

In comparison with other edible legumes, this plant tends to have more and larger nodules with promiscuous inoculation by indigenous nitrogen-fixing bacteria ( May, 1977; National Academy of Science, 1975 ). The bacterium involving in the nodulation of winged bean plant is a *Rhizobium* that nodulates many wild legumes and belongs to the cowpea group ( Thompson and Haryono, 1980 ). Effective tripartite symbiosis (legume- *Rhizobium*-endomycorrhizal fungus ) is essential for desirable growth and nitrogen fixation. However, suitable soil management and fertilization practices are required for high production, mycorrhization and nitrogen fixation due to high levels of soluble iron and aluminum hydrous oxide, high phosphorus fixation, low base cation levels, unfavorable physical structure, and low moisture holding capacity of these soils.

The objectives of these studies were to determine the effects of soil fertility treatments on winged bean growth, nodulation, nitrogenase activity, and nodule composition, and to determine the effect of specific mycorrhiza with and without phosphorus fertilization on growth, nodulation, and nitrogenase activity (  $C_2H_2$  red. ).

## CHAPTER II

### LITERATURE REVIEW

#### Winged Bean

The winged bean ( Psophocarpus tetragonolobus (L) DC ), a twining, herbaceous perennial, can grow to over 3 m length when supported. The leaves are trifoliate, and carried on long stiff erect petiol. The blue, white or purple flowers are self-pollinating. The pods form longitudinal jagged wings and they contain from 5-20 seeds each. The pods develop in two stages with 20 days for ultimate size and 44 days for mature seeds. The globular-shaped, smooth, shiny seeds may be white, brown, black or mottled, and vary in weight from 0.06 to 0.40 g. The roots grow horizontally at shallow depth and become tuberous about 2 months after germination ( Pospisil et al., 1971 ). Excellent nodulation has occurred on the root system of this plant from indigenous Rhizobium without additional inoculation. The individual winged bean plants may develop 400 to 500 nodules with diameters of individual nodules to approximately 1.2 cm and weighing over 0.5 g ( Masfield, 1957 ).

Winged bean crops have been grown in regions that have heavy rainfall during the year. The plant thrives in areas with an annual rainfall of 250 cm or more and may be most productive under irrigation

condition. Winged bean usually is not affected with serious pests and diseases at present, however, the most damaging and widespread pest is the root-knot nematode in Papua New Guinea ( Thompson and Haryono, 1980 ).

The dry seeds contain about 26-45 % protein and 13-20 % oil and dried winged bean tuber composition is about 18-25 % protein ( Cerny and Kordylas, 1971; May 1977; Thompson and Haryono, 1980 ). The winged bean tuber also contains from 27-31 % carbohydrate on fresh weight basis ( Thompson and Haryono, 1980 ). The protein content of immature pods is 15-16 % on a dry weight basis, which is similar to other legumes species ( Thompson and Haryono, 1980 ).

#### The dark red latosol ( Oxisol ) soil

The dark red and red yellow latosol soils ( Ultisols and Oxisols ) having highly weathered, deep, and low fertility status cover the largest area in the Central Brazil. Lopes and Cox ( 1977 ), studying the fertility status of the surface horizon of a board range of these latosols under cerrado vegetation, summarized that these soils are acidic, low extractable bases, low available P and Zn, low CEC, low organic matter content, and with high levels of Fe and Al saturation.

The fertility response of this dark red latosol soils had been investigated under greenhouse conditions with corn, winged bean and Leucaena. The experiments with corn ( Purcino, 1978 ) showed positive response with the application of N and K but not with P. Phosphorus response was observed only in the presence of N and K. However, Phosphorus fertilization was required for growth, nodulation, and enzyme

activities of winged bean ( Purcino, 1978 ), and *Leucaena* ( Lurlarp, 1982 ). Magnesium showed a depressive effect for winged bean growth and nitrogen fixation. Calcium was essential for nodule growth, but not on nodule setting of *Leucaena* ( Lurlarp, 1982 ).

Yield and tuber growth of winged bean was observed on this soil. Lynd et al. ( 1983 ) concluded that PK treatment resulted in pod and seed yield more than double and tuber growth, nodule mass, and nitrogenase activity more than treble compared to the check, nonfertilized plants.

### Mycorrhiza

Symbiotic relationships are established between legumes and soil microorganisms including species of both bacteria and fungi. *Rhizobium* fix atmospheric nitrogen which is then available to the host legume plant ( Hardy et al., 1968 ). Vesicular-arbuscular ( VA ) endomycorrhizal fungi assist legumes in the absorption soil water and inorganic plant nutrients from the soil especially phosphorus ( Mosse, 1973 ). Both *Rhizobium* and VA endomycorrhizal fungi can increase growth and productivity of legumes. Powell and Daniel ( 1978 ) found that white clover inoculated with *Glomus tenuis* showed higher growth, and phosphorus uptake than nonmycorrhizal plants. Smith and Smith ( 1981 ) reported that mycorrhizal alfalfa showed more extensive nodulation, higher rate of nitrogenase activity, and higher phosphorus content than nonmycorrhizal plants. Barea et al. ( 1980 ) indicated that mycorrhiza stimulated growth, and nodulation of alfalfa by *Rhizobium meliloti*, and the magnitude of the increase was inversely correlated with the soluble P content of the soil. Crush ( 1974 ) working with forage legumes

concluded that nonmycorrhizal Centrosema, Stylosanthes, and Trifolium were smaller and had less nodules and nitrogenase activity than did mycorrhizal plants. Daft and El-Giahmi ( 1974 ) found that endogone mycorrhiza colonization and Rhizobium inoculated French beans increased growth, reproduction, number and weight of nodules, acetylene reduction rates, leghaemoglobin, phosphorus, and total protein contents. Daft and El-Giahmi ( 1976 ) also stated that Rhizobium with Glomus sp produced more dry matter and nodulation in peanut plants as well as increased P and N content. Carling et al. ( 1978 ) suggested that inoculated and infected soybean plants showed higher total plant dry weight, nodule dry weight, and nitrogenase activity over singly or noninfected mycorrhizal soybeans. Schenck and Hinson ( 1973 ) discovered that nodulating soybean inoculated with an endogone fungi yielded 53 % more than uninoculated plants. Bagyaraj et al. ( 1979 ) concluded that VA mycorrhiza can greatly assist yield, nodulation, and nitrogen fixation in field grown soybean plants inoculated with Rhizobium.

Rock phosphate is more available to mycorrhiza colonized legumes. According to Powell ( 1979 ) Naru rock phosphate or slowly soluble phosphate was available to mycorrhizal colonized white clover plants but unavailable to nonmycorrhizal plants. Mosse et al ( 1976 ) showed that mycorrhiza colonization with rock phosphate caused more nodulation and nitrogen fixation in Stylosanthes and Centrosema plants. Purcino and Lynd ( 1985 ) pointed out that mycorrhiza in combination with sparingly soluble  $\text{Ca}_3(\text{PO}_4)_2$  increased growth, nodulation, and nitrogenase activity of Stylosanthes scabra.

## Nitrogen fixation

Several environmental factors influence the process of N fixation such as soil pH, nutrition, day length, light intensity and quality, shading, pests, disease, biotic factor, defoliation, and others. This monograph reviews only the influence of phosphorus, potassium, calcium, and trace elements regarding the experiments.

Graham and Rosas ( 1979 ) studied the effects of increased P levels on growth, nodulation, nitrogenase activity, P content of nodule, and P content in plant of common bean. Root dry weight, stem weight, root percent P, stem percent P, leaf percent P, nodule dry weight, nodule percent P, and N (  $C_2H_2$  red ) fixation were increased by increased P levels as linear response.

Purcino ( 1978 ) using a dark red latosol soil from Brazil conducted the study of the effects of P treatment on growth, nodulation, nitrogenase activity, and some nodule enzymes of winged bean. Phosphorus increased shoot growth, nodule number, nodule weight, nitrogenase activity, and the activity of enzymes associated with  $NH_3$  utilization.

Stanley et al ( 1980 ) found large increases in nodulation with high rate of K fertilization of alfalfa, but not of nodule acetylene reduction on gram fresh weight basis. Also, high correlation between acetylene reduction rates and nodule enzyme activities were apparent. They suggested that K induced increases in acetylene reduction and nodule enzyme activities through increased nodule mass.

Mengel and his collaborators ( 1974 ) working with Vicia faba grown in liquid culture has suggested that nitrogenase activity was

increased with increasing K levels in the medium. They concluded that the effect of K on N fixation was on making more carbohydrates available for nodules and providing more abundant supply of reducing electrons and ATP.

Barta ( 1982 ) with short term study ( 3, 6, 10, 14, 18 & 24 days after shoot regrowth ) found that alfalfa supplied with high level of K showed significantly increased shoot dry weight and high rate of acetylene reduction at 10 to 14 days after shoot removal when plants were in vegetative regrowth. However, no significant differences in nodule dry weight, total structural carbohydrates, concentration of starch, and reducing sugars in nodules and acetylene reduction at 24 days after cutting or flowering stage. He also labeled with  $^{14}\text{CO}_2$  at 12 days after shoot cutting and discovered that nodules from high K roots accumulated significantly greater labeled C than low K nodules and also had significantly greater percent labeled C in the amino acid fraction when sampled 1 hour after labeling, but no significant differences were found in  $^{14}\text{C}$  distribution in nodules sampled 24 hours after labeling. Barta concluded that the increased rate of N fixation during alfalfa regrowth in adequately K supplied plants may be the result of greater assimilated transport to nodules and utilization for synthesis of amino acids.

Lowther and Loneragan ( 1968 ) conducted an experiment to study the effects of Ca with various concentrations on growth and nodulation of subterranean clover. High Ca concentration was required for root infection or nodule initiation more than for plant growth. When the nodule was initiated, low Ca concentration did not effect nodule development but did influence plant growth.

Lowther and Adams ( 1970 ) investigated the effect of lime,

copper and phosphorus on the nodulation and growth of white clover. Nodulation, yield of clover dry matter, and percent phosphorus content were increased at all levels of phosphorus by increasing low levels of lime. High levels of lime depressed nodulation, yield of clover, and the uptake of phosphorus at all levels of phosphorus. They stated that high rates of lime reduced clover yield by reducing the plant uptake of phosphorus.

Lynd and co-workers ( 1981 ) worked with hairy vetch in field and greenhouse conditions to study the influences of plant nutrients on growth, nodulation, nitrogenase, and nodule cytosol. Significant increases in both growth and seed yield resulted from P and K combination. P with and without K in combination significantly increased nodulation and nitrogenase activity at anthesis. Nitrogenase activity levels correlated with nodule mass with highly significant increases resulting from K treatment. Increased P levels in factorial combination with K and Ca significantly increased P content of nodule cytosol. The effects of K addition were significant in influence on nitrogenase activity levels and every parameter except cytosol Ca. Cytosol Na was reduced inversely to increased cytosol K content. Growth increased in quadratic response to increased K levels with and without P combination. Nodule weight, nitrogenase and nodule cytosol K significantly increased in linear response with increased K levels without P interaction. Cytosol Ca, Mg, and P were significantly increased with decrease in Na resulting from increased K additions.

Lynd and McNew ( 1982 ) determined the effects of four K levels in factorial combination with P and Ca on growth, nodulation, and nitrogenase activity of narrowleaf vetch grown in greenhouse and field



conditions. Their results indicated that growth as top dry weight significantly increased with the first increment addition, 100 mg K/kg soil, but increased K levels at 200 mg and 300 mg were not apparent with and without P and Ca combinations. However, nodule mass and nitrogenase activity levels increased linearly with each additional applied K level. Effects of P and Ca addition were apparent on nodule mass and nitrogenase activity but not with significant differences.

Gomes et al. (1983 ) reported that higher levels of K were required to maximize parameters associated with biological nitrogen fixation, such as nodules per plants, nitrogenase activity, aminotransferase activity, nodule cytosol, and ureide content, than to cause maximum top growth of soybean plants. Moreover, the combination of P, Ca, and PCa to K levels caused higher production of all parameters. Fertilization with a given element always increased the nodule concentration of that element.

## CHAPTER III

### MATERIALS AND METHODS

These experiments were conducted in the greenhouse and laboratory at Oklahoma State University to study the effects of fertility treatments on winged bean growth and development, nodulation, nitrogenase enzyme activity level, and selected nodule components involved in the metabolism of symbiotic nitrogen fixation with and without mycorrhiza.

Seeds of the winged bean cultivar WB-21-8, "Tinge", originally from Nigeria, were obtained from the Mayaguez Institute of Tropical Agriculture, Puerto Rico and were used throughout the study.

The soil used in these experiments was the epipedon, 20 cm depth, of a dark red latosol ( Typic Eutruster, isohyperthermic, fine kaolinitic ) of Jaiba, Minas Gerais, Brazil. The soil pH was 6.1 with 3.3% organic matter, cation exchange capacity of 25.4 meq/100 g soil of Ca 13.8, Mg 2.5, K 0.2, Na 0.1, available P 7.5 ppm, Fe 680 ppm, Mn 308 ppm, Zn 1.0 ppm,  $SO_4$  and Al < 1.0 ppm, with sand 24.5%, silt 19.5% and clay 56.0%.

The first experiment was 2 levels of P, 0 and 100 ppm, with low soluble  $Ca_3(PO_4)_2$  as the source of phosphorus for 1 kg of soil with and without mycorrhiza. There were 3 series, 8 different aged crops per series, and 2 replications for each treatment. The treatments were  $P_0$  without mycorrhiza,  $P_0$  with mycorrhiza, and  $P_1$  with mycorrhiza. A specific nonseptate endophyte, Glomus fasciculatus ATCC 38848 was used

for mycorrhiza colonization. The Rhizobium inoculum used was L 8. 3502, a peat base commercial culture of the Nitragin Company, Clearwater, Florida 33516.

The second experiment with 2 series was a 4 X 2 factorial with 4 levels P and 2 levels of K replicated 3 times. The 4 levels of phosphorus were designated as  $P_0$ ,  $P_1$ ,  $P_2$ , and  $P_3$  with 0, 100, 200, and 300 ppm using  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  as the source of P. The 2 K levels as  $K_0$ , and  $K_2$  were 0 and 200 ppm from KCl.

The third experiment with 3 replications and 2 series was 2 X 2 X 2 factorial with 2 levels of P, 2 levels of K, and 2 levels of Ca making 8 treatments  $P_0$  and  $P_1$  were symbolized as the 2 levels of phosphorus with 0 and 200 ppm from  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ . The 2 K levels were 0 and 100 ppm as  $K_0$  and  $K_1$  from KCl. Calcium carbonate was used 0 and 6 milliequivalents per 100 grams soil for 2 levels of Ca represented by  $\text{Ca}_0$  and  $\text{Ca}_1$ .

In the fourth experiment there were 2 series, 2 X 4 factorial with 2 levels of P and 4 levels of K, and 3 replications per treatment. The 2 P levels named as  $P_0$  and  $P_1$  were 0 and 200 ppm and obtained from  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ . The 4 levels of potassium as  $K_0$ ,  $K_1$ ,  $K_2$ , and  $K_3$  were 0, 200, 400, and 600 ppm using KCl as the source of K.

The fifth experiment was 2 X 2 X 2 factorial with 2 levels of P, 2 levels of K, and 2 levels of trace elements, 3 replications, and 2 series. The 2 levels of phosphorus from  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  were  $P_0$  and  $P_1$  with 0 and 100 ppm. The 2 K levels as  $K_0$  and  $K_1$  were 0 and 200 ppm using KCl as K fertilizer. The 2 levels of trace elements as  $tr_0$  and  $tr_1$  were 0 and 200 ppm using trace element mixture ( PH salt mixture ). The PH ( Phillips Hart ) salt mixture contained calcium carbonate 30.0%, calcium phosphate 7.5%, cobalt chloride 0.005%, copper sulfate 0.003%,

dipotassium diphosphate 32.2%, ferric citrate 2.75%, manganese sulfate 0.51%, magnesium sulfate 10.2%, potassium iodide 0.08%, sodium chloride 16.7%, and zinc chloride 0.0025%.

The soil was mixed thoroughly with corresponding forms and quantities of fertilizers for 3 replications of each treatment.

Individual winged bean seed was inoculated with 3 ml of Rhizobium leguminosarum, liquid medium, containing more than  $10^8$  viable cells per ml.. These experiments were carried out as a completely randomized design.

In the first experiment the crops were harvested at 30 days old ( 4-5-83 to 5-11-83 ). A week later, the second crop was harvested with 7 days more age than the first crop. And harvesting was continued at 7 day intervals for the eight differential day-age harvests.

The crops of the second experiment were grown for 89 days ( 9-15-83 to 12-12-83 ) and 95 days ( 1-3-84 to 4-10-84 ). In the third experiment, winged bean plants grew for 90 days for both series from 9-15-83 to 12-14-83 and from 1-3-84 to 4-11-84. In the fourth experiment, plants were 88 days old ( 1-3-84 to 4-9-84 ) and 91 days old ( 3-26-84 to 6-27-84 ). The ages of winged bean plants harvested in the fifth experiment were 90 days and 92 days ( 1-13-84 to 4-12-84 and 3-26-84 to 6-26-84 ).

Harvesting of these experiments occurred in the morning between 8-10 a.m.. Plant tops were clipped, oven dried at 105°C for 24 hours and weighed. The nodule-root systems were carefully shaken free of soil, washed, blotted with paper toweling to remove excess water, and placed in serum cap bottles for nitrogenase ( EC. 1.7.99.2 ) activity ( $C_2H_2$ ) reduction determinations.

Nitrogenase activity was measured according to the method described by Hardy et al. ( 1968 ). The nodules and roots were incubated for one hour with 0.1 atm  $C_2H_2$  at 27°C and  $C_2H_4$  production was detected with a Perkin Elmer GC 3920 with 1.83 mm X 3.2 mm Poropak N 80/100 column. The ethylene standard utilized for calibration and monitoring of gas chromatography analysis was the Scott Ev. Tech, 1090 ppm  $\pm$  5%  $C_2H_4/N_2$ .

After the  $C_2H_4$  reduction analysis, the nodules were picked from the roots, counted, and weighed. Roots were oven dried at 105°C for 24 hours and weighed.

Nodule cytosol composition analyses by the method of Vance et al. ( 1979 ) was slightly modified to separate the cell free nodule extract. Samples of nodule were crushed within glass tubes at 0-5°C with the addition of 0.1 M phosphate buffer ( pH 7.41 ) in the ratio of 10 ml of solution to each gram of nodule. The filtered homogenate was subjected to ultrasonic 7.3 pulse frequency in an ice bath for 30 seconds using a PT W ST Williams Polytron ( Brinkman Instruments, Inc ) followed by refrigerated centrifugation at  $12 \times 10^3$  g for 10 minutes. The clear, cell-free nodule extract ( cytosol ) was aseptically transferred to sterile culture tubes and stored at 0-5°C. The nodule components were determined using a Perkin-Elmer 373 Atomic Absorption Flame Spectrophotometer with K, Ca, Fe, and Mg in lanthanum chloride ( 0.1 N HCl ) solution and Na without lanthanum chloride addition. Aspartate transaminase enzyme activity levels were determined as International Units (IU) that induce transformation of 1.0  $\mu$  mole of specific substrate per minute determined in 3.0 ml of reaction volume, 1 cm light path, 27°C ( Bergmeyer, 1978 ). Ureide composition was determined as total

ureide-glycolates that included allantoinates and glyoxolurea catabolites ( Trijibels, F. and G. D. Vogels., 1966 ). Non-conjugate and inorganic P were determined with the ascorbic acid oxidation method as phosphomolybdenum blue. The second series of each experiment was repeated without addition of any fertilizers with the same procedures.

General linear module analysis of variance was used for significant difference determination within parameters by treatment. The data obtained were analyzed according to the Statistical Analysis System ( SAS ) using the PROCEDURE ANOVA for single degree of freedom comparisons for fertility treatments, PROCEDURE CORR to determine possible relationship between paired independent variable.

## CHAPTER IV

### RESULTS AND DISCUSSIONS

The effects of endomycorrhizal colonization with and without P fertilization on winged bean growth, nodulation, and nitrogenase activity are presented in Table I.

The significantly highest root and nodule weight was 2.66 g plant<sup>-1</sup> of the endomycorrhiza treatment. The mycorrhiza with slowly soluble phosphorus showed significantly lower root and nodule fresh weight of 1.94 g plant<sup>-1</sup> than the check with LSD .05 of 0.25 g.

Number of nodules significantly increased with the endomycorrhiza treatment. However, mycorrhiza with sparingly soluble phosphorus caused nonsignificantly lower number of nodules than the control. The significantly highest number of nodules with 19 nodules plant<sup>-1</sup> resulted from endomycorrhiza with LSD .05 of 1.84 nodules.

Fresh nodule weight increased significantly due to mycorrhiza with and without slowly soluble phosphorus. The lowest nodule mass was 0.10 g plant<sup>-1</sup> due to the check and the highest of 0.19 g plant<sup>-1</sup> occurred with endomycorrhiza with slowly soluble P. The LSD .05 was 0.03 g.

The significantly highest root dry weight was 0.21 g plant<sup>-1</sup> resulting from mycorrhiza treatment, LSD .05 of 0.03 g. The addition of slowly soluble P to mycorrhiza significantly showed lower root dry

TABLE I  
EFFECTS OF MYCORRHIZA WITH AND WITHOUT P ON GROWTH,  
NODULATION, AND NITROGENASE ACTIVITY OF WINGED  
BEAN GROWN ON A TYPIC EUTRUSTOX

Parameter	Treatments				
	0	Myz	Myz+P	Ave	LSD .05
Root & nodule g.	2.35	2.66	1.94	2.32	0.25
Nodule number	11	19	10	13	1.84
Nodule fr. wt. g.	0.11	0.15	0.19	0.15	0.03
Root dry wt. g.	0.21	0.21	0.17	0.19	0.03
Stem dry wt. g.	0.24	0.32	0.34	0.30	0.04
Leaf dry wt. g.	0.29	0.36	0.38	0.35	0.04
Stem length cm.	131.87	156.41	162.91	150.39	10.91
Top fr. wt. g.	3.29	4.08	4.89	4.08	0.33
Nase u mole.	11.89	19.56	33.76	21.73	5.14

Each figure is pooled age of each treatment.

Nase = Nitrogenase activity as  $C_2H_4$  u mole  $g^{-1}$  nodule  $h^{-1}$ .

Applied plant nutrients were 0 and 100 mg P  $Kg^{-1}$  soil.

Endomycorrhiza inoculant was Glomus fasciculatum ATCC 38848.



weight,  $0.17 \text{ g plant}^{-1}$  than the control of  $0.20 \text{ g plant}^{-1}$ .

Stem dry weight showed significant increases due to mycorrhiza with and without sparingly soluble phosphorus. Mycorrhiza with phosphorus gave the significantly highest stem dry weight of  $0.34 \text{ g plant}^{-1}$  with LSD .05 of  $0.04 \text{ g}$ .

Significant increases in leaf dry weight resulted with mycorrhiza and mycorrhiza with slowly soluble phosphorus. The significantly highest leaf dry weight of  $0.38 \text{ g plant}^{-1}$  was with the combination of mycorrhiza and slowly soluble phosphorus. The check had the lowest leaf dry weight of  $0.29 \text{ g plant}^{-1}$ . The level of significance at .05 was  $0.038 \text{ g}$ .

Stem length showed significant increases due to endomycorrhiza and endomycorrhiza with slowly soluble phosphorus. The significantly longest stem was  $162.91 \text{ cm plant}^{-1}$  with the endomycorrhiza and slowly soluble phosphorus with the shortest of  $131.87 \text{ cm}$  from the control. The LSD .05 was of  $10.91 \text{ cm}$ .

The lowest top fresh weight of  $3.29 \text{ g plant}^{-1}$  was from the check and highest of  $4.89 \text{ g plant}^{-1}$  was from endomycorrhizal fungi with slowly soluble phosphorus. The level of significant difference at .05 was  $0.33 \text{ g}$ . The top fresh weight was significantly increased by endomycorrhiza with and without slowly soluble phosphorus.

Nitrogenase activity was generally analogous to nodule mass response to the treatments. Significantly highest nitrogenase activity of  $33.76 \text{ u mole C}_2\text{H}_4 \text{ g}^{-1} \text{ nodule h}^{-1}$  occurred with endomycorrhiza and slowly soluble  $\text{Ca}_3(\text{PO}_4)_2$  treatment. The lowest of  $11.98 \text{ u mole C}_2\text{H}_4 \text{ g}^{-1} \text{ nodule h}^{-1}$  occurred with the control. Significant increases in nitrogenase activity resulted from endomycorrhizal inoculation with slowly soluble P treatment and endomycorrhiza. LSD .05 was  $5.14 \text{ u mole}$

$C_2H_4$  g<sup>-1</sup> nodule h<sup>-1</sup>.

The effects of plant age on growth, nodulation, and nitrogenase activity of winged bean are summarized in Table II.

The significantly highest fresh nodule and root weight was 2.73 g plant<sup>-1</sup> when winged bean was 77-79 days old and the lowest was 1.86 g plant<sup>-1</sup> at 42-44 days of age with LSD .05 of 0.61 g. Generally, root and nodule fresh weight consistently increased with the plant age.

Number of nodules did not significantly change as plant age increased. The highest nodule number was 14 nodules plant<sup>-1</sup> at 63-65 and 70-72 days of age with the lowest of 11 nodules plant<sup>-1</sup> at 49-51 days of age. The .05 level of significance was 2.41 nodules plant<sup>-1</sup>.

The significantly highest fresh nodule weight with LSD .05 of 0.06 g was 0.18 g plant<sup>-1</sup> at 70-72 growing days and the lowest fresh nodule weight was at 42-44 days old and 49-51 days old with 0.11 g plant<sup>-1</sup>. Fresh nodule weight was increased in quadratic response with plant age. The fresh nodule weight was decreased from 0.18 g plant<sup>-1</sup> as the highest fresh nodule weight to 0.16 g plant<sup>-1</sup> after 70-72 days old to 77-79 days of age.

Linear increases in root growth resulted with increased plant age from 0.16 g plant<sup>-1</sup> to 0.25 g plant<sup>-1</sup> at 42-44 days to 77-79 days. The significantly largest root dry weight was 0.25 g plant<sup>-1</sup> at 77-79 days of age with the smallest of 0.16 g plant<sup>-1</sup> at 42-44 days old. The .05 level of significant difference was 0.67 g.

The significantly highest stem growth was obtained when winged bean was 77-79 days old with 0.34 g plant<sup>-1</sup>. The lowest was 0.21 g plant<sup>-1</sup> resulting from 42-44 and 49-51 days of growth. The LSD .05 of stem dry weight was 0.11 g. Stem dry weight generally increased linearly

TABLE II  
EFFECTS OF PLANT AGE ON GROWTH, NODULATION, AND  
NITROGENASE ACTIVITY OF WINGED BEAN  
GROWN ON A TYPIC EUTRUSTOX

Parameter	Day						LSD.05
	42-44	49-51	56-58	63-65	70-72	77-79	
Root and nodule g.	1.86	1.99	2.24	2.18	2.28	2.73	0.61
Nodule Weight g.	0.11	0.11	0.13	0.14	0.18	0.16	0.06
Nodule number	14	11	13	14	14	13	2.41
Root dry wt g.	0.16	0.17	0.18	0.20	0.21	0.25	0.07
Stem dry wt g.	0.21	0.21	0.29	0.27	0.32	0.34	0.11
Leaf dry wt g.	0.27	0.28	0.35	0.35	0.36	0.37	0.09
Stem length cm	117.9	117.4	137.9	151.4	145.0	159.2	35.82
Top fresh weight g.	3.20	3.43	4.29	3.99	4.11	4.42	1.00
Nase u mole	21.2	20.2	21.3	15.5	19.0	15.6	5.47

Each figure is pooled treatment of each age.

Nase = Nitrogenase activity as  $C_2H_4$  u mole  $g^{-1}$  nodule  $h^{-1}$

The endomycorrhiza inoculant was Glomus fasciculatum ATCC 38848.

from 0.21 g plant<sup>-1</sup> to 0.34 g plant<sup>-1</sup> as the winged bean plants matured from 42-44 to 77-79 days of age.

Linear growth of leaf from 0.27 g plant<sup>-1</sup> as the lowest leaf dry weight to 0.37 g plant<sup>-1</sup> as the significantly highest leaf dry weight occurred with the increased plant age from 42-44 to 77-79 days of growth. The 0.09 g plant<sup>-1</sup> was the .05 level of significant difference for leaf dry weight.

The significantly highest stem length with 159.2 cm was obtained when the winged bean was 77-79 days old. The lowest stem length of 117.4 cm at 49-51 days of age. The LSD .05 of stem length was 35.82 cm. However, the stem length increased as a linear response with increased plant age from 117.9 to 159.2 cm at 42-44 to 77-79 days of age.

Top growth was generally increased with plant age from 3.20 g plant<sup>-1</sup> as the lowest top fresh weight to 4.42 g plant<sup>-1</sup> as the significantly highest top fresh weight at 42-44 days old and 77-79 days old, respectively. The level of significance at .05 was 1.00 g.

The significantly highest nitrogenase activity was observed at 56-58 days of age with 21.3 u mole C<sub>2</sub>H<sub>4</sub> g<sup>-1</sup> nodule h<sup>-1</sup> with the lowest of 15.6 u mole C<sub>2</sub>H<sub>4</sub> g<sup>-1</sup> nodule h<sup>-1</sup> at 63-65 days of age. Generally, the nitrogenase activity level decreased with increased plant age from 42-44 days to 77-79 days. The level of significant difference at .05 for nitrogenase activity was 5.47 u mole C<sub>2</sub>H<sub>4</sub> g<sup>-1</sup> nodule h<sup>-1</sup>.

Influences of endomycorrhiza with and without P on growth, nodulation, and nitrogenase activity are shown on Table III.

Generally, mycorrhiza showed the highest root and nodule fresh weight at each plant age except at 56-58 days of age. The significantly highest root and nodule fresh weight caused by mycorrhiza at 70-72 days

TABLE III  
EFFECTS OF MYCORRHIZA WITH AND WITHOUT P ON GROWTH,  
NODULATION, AND NITROGENASE ACTIVITY OF WINGED  
BEAN GROWN ON A TYPIC EUTRUSTOX

Parameter	Soil Treatment, Plant Age ( Days )																	
	42-44			49-52			56-58			63-65			70-72			77-79		
	O	M	MP	O	M	MP	O	M	MP	O	M	MP	O	M	MP	O	M	MP
RN	.20	.22	.17	.20	.30	.13	.24	.19	.23	.26	.23	.16	.21	.35	.21	.26	.33	.21
NN	10	17	12	6	18	9	12	17	10	14	23	8	13	26	11	12	17	9
NW	.07	.09	.01	.05	.09	.01	.08	.01	.01	.14	.19	.12	.10	.33	.22	.14	.15	.17
RW	.20	.19	.12	.17	.24	.17	.20	.14	.20	.20	.18	.13	.21	.28	.19	.23	.25	.29
SW	.23	.20	.21	.13	.25	.21	.28	.29	.36	.27	.33	.25	.24	.63	.38	.29	.38	.33
LW	.29	.27	.28	.23	.31	.28	.31	.33	.42	.31	.41	.30	.26	.54	.43	.31	.45	.35
SL	131	112	139	92	131	119	127	146	169	169	153	149	133	219	183	148	172	155
TW	31	30	38	23	35	34	32	38	55	37	49	40	29	55	56	38	46	47
NS	8	14	47	9	15	29	9	14	37	15	11	20	11	37	25	11	16	17

RN = Root & Nodule (g)

NN = Nodule Number

NW = Nodule Weight (g)

RW = Root Dry Weight (g)

SW = Stem Dry Weight (g)

LW = Leaf Dry Weight (g)

SL = Stem Length (cm)

TW = Top Fresh Weight (g)

NS = Nitrogenase Activity ( $C_2H_4$   $\mu$  mole  $g^{-1}$  nodule  $h^{-1}$ )

Applied plant nutrients were 0 and 100 mg P  $kg^{-1}$  soil.

The endomycorrhiza inoculant Glomus fasciculatum ATCC 38848.

of age of  $3.6 \text{ g plant}^{-1}$  with the lowest of  $1.6 \text{ g plant}^{-1}$  from mycorrhiza plus slowly soluble phosphorus at 63-65 days of age with LSD .05 of 0.25 g.

Nodule number increased at each plant age with mycorrhiza treatment. The significantly highest nodule number resulted from mycorrhiza at 70-72 days old and the lowest number of nodules resulted from mycorrhiza at 42-44 days old. The highest and lowest nodule number were 26 and 17 nodules  $\text{plant}^{-1}$ , respectively. The LSD .05 was 1.84 nodules  $\text{plant}^{-1}$ .

Mycorrhiza resulted in the highest fresh nodule weight at each plant age except at 56-58 and 77-79 days of age. The significantly highest fresh nodule weight was  $0.33 \text{ g plant}^{-1}$  with endomycorrhiza treatment at 70-72 days old. The lowest fresh nodule weight was  $0.01 \text{ g plant}^{-1}$  of mycorrhiza with sparingly soluble phosphorus at 42-44, 49-51, and 56-58 days old, respectively. The level of significant difference at 0.5 was 0.03 g.

Mycorrhiza resulted in the highest root dry weight at 49-57 and 70-72 days of age. Mycorrhiza with slowly soluble P resulted in the highest root dry weight at 56-58 and 77-79 days of age. The significantly highest root dry weight,  $0.29 \text{ g plant}^{-1}$  occurred with mycorrhiza with slowly soluble P at 77-79 days old with 0.03 g as LSD .05. The lowest root dry weight was  $0.13 \text{ g plant}^{-1}$  with mycorrhiza with sparingly soluble P at 63-65 days old.

Mycorrhiza showed the highest stem dry weight at 49-51, 63-65, 70-72, and 77-79 days of age. At 42-44 and 56-58 days of age, the highest stem dry weight was obtained with the control and mycorrhiza with sparingly soluble phosphorus, respectively. The significantly highest

stem dry weight with LSD .05 of 0.04 g was with endomycorrhiza at 70-72 days old of 0.63 g plant<sup>-1</sup> with the lowest of 0.13 g plant<sup>-1</sup> from the control at 49-51 days of age.

The significantly highest leaf dry weight was caused by mycorrhiza treatment at 49-51, 63-65, 70-72, and 77-79 days old. Mycorrhiza with slowly soluble phosphorus resulted in highest leaf dry weight at 49-51 days of age. The significantly highest leaf dry weight was 0.54 g plant<sup>-1</sup> with mycorrhiza at 70-72 days old. The lowest leaf dry weight was 0.13 g plant<sup>-1</sup> from the control at 49-51 days of age with LSD .05 of 0.04 g.

The longest stem length, 219 cm was from the mycorrhiza treatment at 70-72 days of age with the shortest of 92 cm with the control at 49-51 days of age with 10.91 cm as the LSD .05. Mycorrhiza resulted in the longest stem length at 49-51, 63-65, 70-72, and 77-79 days of age. Endomycorrhiza with slowly soluble phosphorus resulted in longest stem length at 49-51 days old.

Top fresh weight was highest with mycorrhiza at 49-51 and 63-65 days of age. Mycorrhiza with slowly soluble P showed the highest fresh top weight at 42-44, 56-58, 70-72, and 77-79 days of age. The highest and lowest top fresh weight were 5.6 and 2.3 g plant<sup>-1</sup> from mycorrhiza with sparingly soluble P at 70-72 days old and the check at 49-51 days old, respectively. The .05 level of significance was 0.33 g.

Generally, the nitrogenase activity was highest with mycorrhiza with slowly soluble P except at 70-72 days old. The significantly highest nitrogenase activity of 47 u mole C<sub>2</sub>H<sub>4</sub> g<sup>-1</sup> nodule h<sup>-1</sup> was from mycorrhiza with slowly soluble phosphorus at 42-44 days old with the lowest nitrogenase activity of 8 u mole C<sub>2</sub>H<sub>4</sub> g<sup>-1</sup> with the control at the

same plant age. The level of significant difference at .05 was 5.14  $\mu$  mole  $\text{C}_2\text{H}_4$   $\text{g}^{-1}$  nodule  $\text{h}^{-1}$ .



Effects of four P levels in factorial combination with K on growth, nodulation, and nitrogenase activity levels of winged bean are shown in Table IV.

Leaf dry weight increased in linear response to increased P levels from 0.89 g plant<sup>-1</sup> of mean P<sub>0</sub> to 1.84 g plant<sup>-1</sup> of mean P<sub>3</sub>. However, the effects of K decreased the average of leaf dry weight as K<sub>0</sub> of 1.50 g plant<sup>-1</sup> and K<sub>1</sub> of 1.36 g plant<sup>-1</sup>. The significantly highest leaf dry weight of 1.92 g plant<sup>-1</sup> occurred from P<sub>3</sub>K treatment with the lowest of 0.75 g plant<sup>-1</sup> from the K treatment. The level of significance at .05 was 0.26 g plant<sup>-1</sup>.

Highest stem dry weight was obtained from the P<sub>3</sub> treatment with 1.88 g plant<sup>-1</sup> and the lowest stem dry weight was 0.78 g plant<sup>-1</sup> from K treatment. A quadratic response was apparent with increased pooled P levels. The K effect resulted in slight stem growth decreases when K treatment was combined with P levels, 1.41 g plant<sup>-1</sup> compared to 1.47 g plant<sup>-1</sup> of no K addition. The .05 level of significance was 0.16 g plant<sup>-1</sup>.

A quadratic increases in root dry weight occurred with the increased pooled P levels. Slightly lower root dry weight resulted from the K effects. The P<sub>3</sub> treatment showed the highest root dry weight with 0.68 g plant<sup>-1</sup> in comparison with the lowest of 0.40 g plant<sup>-1</sup> from K treatment. The 0.11 g plant<sup>-1</sup> was the .05 level of significance.

Linearly increased fresh nodule weight occurred from the increased pooled P levels from 0.60 g plant<sup>-1</sup> to 1.63 g plant<sup>-1</sup>. The significantly highest fresh nodule weight was 1.81 g plant<sup>-1</sup> from the P<sub>3</sub>K treatment with the lowest of 0.47 g plant<sup>-1</sup>. There was no fresh nodule weight increase due to the K effects.

TABLE IV  
EFFECTS OF P LEVELS WITH AND WITHOUT K ON GROWTH, NODULATION,  
AND NITROGENASE ACTIVITY OF WINGED BEAN GROWN  
ON A TYPIC EUTRUSTOX, SERIES I

Parameter	Treatment	0	P <sub>1</sub>	P <sub>2</sub>	P <sub>3</sub>	Ave
Leaf wt g.	0	1.02	1.78	1.43	1.76	1.50
	K	0.75	0.88	1.90	1.92	1.36
	Ave	0.89	1.33	1.67	1.84	(0.26)
Stem wt g.	0	1.00	1.43	1.33	1.88	1.47
	K	0.78	1.65	1.68	1.51	1.41
	Ave	0.89	1.54	1.51	1.70	(0.16)
Root wt g.	0	0.48	0.63	0.46	0.68	0.56
	K	0.40	0.65	0.63	0.51	0.55
	Ave	0.44	0.64	0.55	0.60	(0.11)
Nodule wt g.	0	0.47	1.37	1.45	1.45	1.19
	K	0.73	0.64	1.57	1.81	1.19
	Ave	0.60	1.01	1.57	1.63	(0.23)
Nodule No	0	43	54	29	22	37
	K	37	23	29	32	30.2
	Ave	40	38.5	29	27	(5.26)
Nitrogenase C <sub>2</sub> H <sub>4</sub> u mole g <sup>-1</sup> nodule h <sup>-1</sup>	0	16.0	57.0	77.5	140.0	72.6
	K	36.0	9.0	143.5	124.0	78.1
	Ave	26.0	33.0	110.5	132.0	(5.45)

Applied plant nutrient levels as mg P kg<sup>-1</sup> soil were 0, 100, 200, and 300 and mg K kg<sup>-1</sup> soil were 0, and 200 with analysis of variance as General Linear Model, LSD .05 in parenthesis of each parameter.

The P levels increased number of nodule as quadratic response with the highest nodule number of 43 nodules plant<sup>-1</sup> and the lowest nodule number of 22 nodules plant<sup>-1</sup>. The K addition decreased the average number of nodule plant<sup>-1</sup> from 37 of K<sub>0</sub> to 30 of K<sub>1</sub> nodules plant<sup>-1</sup>.

Highest nitrogenase activity of 143.5 C<sub>2</sub>H<sub>4</sub> u mole g<sup>-1</sup> nodule h<sup>-1</sup> was with the P<sub>2</sub>K treatment and was significantly higher than the lowest of 9.0 C<sub>2</sub>H<sub>4</sub> u mole g<sup>-1</sup> nodule h<sup>-1</sup> from the P<sub>1</sub>K treatment. The pooled P levels linearly increased the nitrogenase activity of 26 to 132 C<sub>2</sub>H<sub>4</sub> u mole g<sup>-1</sup> nodule h<sup>-1</sup>. K also increased slightly the nitrogenase activity from 72.6 to 78.1 C<sub>2</sub>H<sub>4</sub> u mole g<sup>-1</sup> nodule h<sup>-1</sup>.

Plant nutrients were applied to the first experiment, Series I. The Series II was conducted for residual effects from the added plant nutrients of Series I. The effects of the residual plant nutrients on growth, nodulation, and nitrogenase activity levels of winged bean are presented in Table V.

Higher leaf dry weight occurred when P levels with and without K treatment combination were applied. The P<sub>1</sub>K treatment gave the significantly highest leaf dry weight yield of 1.07 g plant<sup>-1</sup>, whereas, the lowest leaf dry weight was 0.20 g plant<sup>-1</sup> from the check. Higher leaf dry weight yields resulted from the K addition with P levels, 0.74 g plant<sup>-1</sup> compared to K<sub>0</sub> of 0.46 g plant<sup>-1</sup>. The effects of the increased pooled P levels was quadratic. The LSD .05 was 0.18 g.

A yield of 1.15 g plant<sup>-1</sup> was the significantly highest stem dry weight with P<sub>1</sub>K treatment in comparison to the lowest of the control of 0.25 g plant<sup>-1</sup>. P levels with K treatment combination resulted in higher stem dry weight, 0.85 g plant<sup>-1</sup> compared to K<sub>0</sub> of 0.37 g plant<sup>-1</sup>. A

TABLE V  
EFFECTS OF RESIDUAL P LEVELS WITH AND WITHOUT K ON GROWTH,  
NODULATION, AND NITROGENASE ACTIVITY OF WINGED  
BEAN GROWN ON A TYPIC EUTRUSTOX, SERIES II

Parameter	Treatment	0	P <sub>1</sub>	P <sub>2</sub>	P <sub>3</sub>	Ave
Leaf wt g.	0	0.20	0.63	0.63	0.37	0.46
	K	0.29	1.07	0.73	0.88	0.74
	Ave	0.25	0.85	0.68	0.63	(0.18)
Stem wt g.	0	0.25	0.50	0.43	0.30	0.37
	K	0.45	1.15	0.77	1.03	0.85
	Ave	0.35	0.83	0.60	0.67	(0.17)
Root wt g.	0	0.27	0.19	0.13	0.12	0.18
	K	0.35	0.33	0.31	0.20	0.28
	Ave	0.31	0.26	0.22	0.16	(0.04)
Nodule wt g.	0	0.31	0.43	0.39	0.29	0.34
	K	0.26	1.17	1.12	1.17	0.93
	Ave	0.29	0.80	0.78	0.73	(0.21)
Nodule No.	0	17	12	13	14	14
	K	20	39	40	49	37
	Ave	18	25	26	31	(7.73)
Nitrogenase C <sub>2</sub> H <sub>4</sub> u mole g <sup>-1</sup> nodule h <sup>-1</sup>	0	25.6	38.6	40.0	53.0	38.8
	K	25.0	61.6	59.0	117.6	65.8
	Ave	25.3	50.1	49.5	85.3	(8.09)

Residual of applied plant nutrient levels as mg P kg<sup>-1</sup> soil were 0, 100, 200, and 300 and mg K kg<sup>-1</sup> soil were 0, and 200 with analysis of variance as General Linear Model, LSD .05 in parenthesis for each parameter.

quadratic response was apparent with increased pooled P levels with LSD .05 of 0.17 g.

Root dry weight was decreased when P levels were applied with and without K combination, 0.31 g for  $P_0$  to 0.16 g with  $P_3$  treatment. K addition of 0.28 g was significantly higher than  $K_0$  of 0.18 g. LSD .05 was 0.04 g.

Pooled P levels indicated a linear response in fresh nodule weight from 0.29 g plant<sup>-1</sup> to 0.73 g plant<sup>-1</sup>. The effect of K addition resulted in significantly higher fresh nodule weight with  $K_1$  of 0.93 g plant<sup>-1</sup> and  $K_0$  of 0.34 g plant<sup>-1</sup>. The significantly highest fresh nodule weight, 1.17 g plant<sup>-1</sup> resulted with both  $P_1K$  and  $P_3K$  treatments. Lowest yield was from  $P_3K$  treatment of 0.29 g. The LSD .05 was 0.21 g.

The number of nodule linearly increased from 18 nodules plant<sup>-1</sup> to 31 nodules plant<sup>-1</sup> with the increased P levels. The K effect caused higher number of nodule of 37 compared to  $K_0$  of 14 nodules plant<sup>-1</sup>. The  $P_3K$  resulted in significantly highest number of nodules of 49 nodules plant<sup>-1</sup>, lowest of 12 nodules per plant with  $P_1K$  treatment. The .05 level of significance was 7.73 nodules plant<sup>-1</sup>.

Quadratic response in nitrogenase activity with increased pooled P levels ranged from 24.3 to 85.3  $C_2H_4$  u mole g<sup>-1</sup> nodule h<sup>-1</sup>. The nitrogenase activity of 117.6  $C_2H_4$  u mole g<sup>-1</sup> nodule h<sup>-1</sup> was the significantly highest nitrogenase activity level with  $P_3K$  treatment, whereas, the K treatment showed the lowest nitrogenase activity of 25  $C_2H_4$  u mole g<sup>-1</sup> nodule h<sup>-1</sup>. K addition significantly increased  $C_2H_4$  production 65.8 u mole compared to the pooled  $K_0$  average of 38.8 u mole. The level of significance at .05 was 8.09 u mole g<sup>-1</sup> nodule h<sup>-1</sup>.

Correlation coefficients of measured parameters as influenced by

soil treatments of P levels with and without K combination for Series I ( fertilized ) and Series II ( residual ) are summarized in Table VI.

Significant correlation coefficients were apparent with Series I for nitrogenase X stem (0.568\*), nitrogenase X leaf (0.781\*), and nitrogenase X nodule weight (0.815 \*\*); for nodule weight X stem (0.618\*), nodule weight X leaf (0.902\*\*), and nodule weight X root (0.392\*); for root X stem (0.837\*\*) and root X leaf (0.539\*); and for leaf X stem (0.617\*).

Significant correlation coefficients were apparent for residual effects in Series II for nitrogenase X stem (0.670\*), nitrogenase X leaf (0.621\*), nitrogenase X nodule number (0.697\*), and nitrogenase X nodule weight (0.731\*). Correlations were attained with nodule weight X stem (0.913\*\*), nodule weight X leaf (0.860\*\*), nodule weight X root (0.405\*), and nodule weight X nodule number (0.854\*\*). Nodule number was correlated with stem (0.800\*), leaf (0.709\*), and root (0.496\*). Root was correlated with stem (0.388\*) and leaf was correlated with stem (0.911\*\*).

TABLE VI

CORRELATION COEFFICIENTS OF PLANT GROWTH PARAMETERS, NITROGENASE,  
AND NODULATION OF WINGED BEAN AS AFFECTED BY P LEVELS  
APPLIED WITH AND WITHOUT K ADDITION

Parameter	Series I					
	Stem	Leaf	Root	Nod No	Nod wt	Nase
Stem		0.6712*	0.8371**	0.0828	0.6176*	0.5681*
Leaf	0.9110**		0.5390*	0.2044	0.9017**	0.7809*
Root	0.3875*	0.2538		0.3126	0.3920*	0.2589
Nod No	0.8001*	0.7090*	0.4954*		-0.0346	-0.3644
Nod Wt	0.9132**	0.8598**	0.4050*	0.8542**		0.8145**
Nase	0.6704*	0.6211*	-0.0796	0.6977*	0.7316*	
Series II						

\*, \*\* = Significant at P = 0.05 and 0.01, respectively  
Plant nutrients applied for Series I  
Residual response of applied nutrients for Series II

Effects of applied P levels with and without K treatment combination on nodule cytosol compositions of P, K, Na, Ca, Mg, aspartate transaminase, and ureide are summarized in Table VII.

Linearly increased nodule cytosol P content resulted from increased pooled P levels with the significantly highest of 408 ug/g nodule from  $P_3K$  treatment and the lowest of 74 ug/g nodule from the  $K_0$  treatment. A lower cytosol P content was 258.8 ug/g nodule with  $K_1$  treatment compared to 321.5 ug/g nodule with  $K_0$  treatment. The level of significance at .05 was 19.5 ug/g.

The K content of nodule cytosol increased in linear response with the increased pooled P levels from 1743 ug/g to 2473 ug/g. However, K addition resulted in higher K content with 2862.5 ug/g for  $K_1$  treatment and 1472.5 ug/g for  $K_0$  treatment. The control with 1310 ug/g nodule was the lowest K content and the significantly highest K content occurred from the  $P_3K$  treatment with 3420 K ug/g nodule. LSD .05 was 73.5 ug/g.

A quadratic response in Na cytosol content of nodule was apparent with the increased P levels. The effect of K caused the decrease in Na uptake of 95 ug/g compared to  $K_0$  of 177.5 ug/g. The  $P_1K$  and  $P_2K$  treatments with 185 ug/g nodule was the significantly highest K cytosol content with 9.30 ug/g as the LSD .05.

Calcium uptake increased with increasing pooled P levels 69 to 81 ug/g and increased with K addition of 74.4 ug/g with  $K_0$  of 70.3 ug/g. The significantly highest Ca content of 104 ug/g nodule occurred from the  $P_3K$  treatment with the lowest of 51 ug/g of  $K$  treatment. The .05 significant level of Ca content was 10.6 ug/g.

A quadratic response occurred with Mg content due to the increased P levels. The effect of K addition caused lower Mg uptake from



TABLE VII  
EFFECTS OF P AND K FACTORIAL COMBINATIONS ON COMPOSITION OF  
NODULE CYTOSOL OF WINGED BEAN GROWN ON A  
TYPIC EUTRUSTOX, SERIES I

Parameter	Treatment	0	P <sub>1</sub>	P <sub>2</sub>	P <sub>3</sub>	Ave
P ug/g	0	190	276	416	404	321.5
	K	74	252	300	408	258.8
	Ave	132	264	358	406	(19.5)
K ug/g	0	1310	1490	1565	1525	1472.5
	K	2175	3030	2825	3420	2862.5
	Ave	1743	2260	2195	2473	(73.5)
Na ug/g	0	175	185	185	165	177.5
	K	80	135	85	80	95.0
	Ave	128	160	135	123	(9.3)
Ca ug/g	0	87	62	74	59	70.3
	K	51	78	102	104	74.4
	Ave	69	70	88	81	(10.6)
Mg ug/g	0	155	185	130	85	138.8
	K	48	78	50	128	75.8
	Ave	102	131	90	106	(13.9)
Aspartate Transaminase IU/g	0	20	40	116	83	64.5
	K	10	108	78	117	78.0
	Ave	15	74	97	100	(4.4)
Ureide u mole/g	0	82	62	107	52	75.5
	K	45	64	77	52	59.3
	Ave	63	63	92	52	(5.2)

Applied plant nutrient levels as mg P kg<sup>-1</sup> soil were 0, 100, 200, and 300 and mg K kg<sup>-1</sup> soil were 0, and 200 with analysis of variance as General Linear Model, LSD .05 in parenthesis of each parameter.

138.8 of  $K_0$  to 75.8 ug/g. The  $P_1$  treatment with 13.9 ug/g as LSD .05 was significantly highest Mg content, 185 ug/g and the lowest of 50 ug/g of  $P_2K$  treatment.

Aspartate transaminase increased linearly from 15 IU/g nodule to 100 IU/g nodule with increasing pooled P levels. Significantly increased aspartate transaminase occurred with K addition from 64.5 of  $K_0$  to 78 IU/g. The significantly highest aspartate transaminase of 117 IU/g nodule was from  $P_3K$  treatment and the lowest of 10 IU/g with  $P_0K$  treatment with 4.4 IU/g as LSD .05.

The  $P_2$  treatment gave the significantly highest ureide content of 107 u moles/g nodule with the lowest of 52 u moles/g with  $P_3$  and  $P_3K$  treatments. The effect of K addition resulted in lower ureide content from 75.5 u moles/g of  $K_0$  treatment to 59.3 u moles/g as  $K_1$  treatment. Increased ureide content was quadratic with increased P levels. The .05 level of significance was 5.2 u moles/g nodule.

Correlation coefficients of measured cytosol P, K, Na, Ca, Mg content, aspartate transaminase, and ureide of nodule as influenced by soil treatments of P levels with and without K addition are shown in Table VIII.

Highly significant correlation coefficient was shown for P X aspartate transaminase (0.664\*\*). Negative significant correlation were observed with K X Na (-0.534\*\*) and aspartate transaminase X Mg (-0.416\*). Also, ureide was significantly correlated with Ca content of cytosol nodule (0.380\*).

TABLE VIII  
CORRELATION COEFFICIENTS FOR CYTOSOL CONTENT OF NODULE  
OF WINGED BEAN AS AFFECTED BY P LEVELS APPLIED  
WITH AND WITHOUT K ADDITION

Parameter	P	K	Na	Ca	Mg	ALT	URD
P		0.1729	0.0508	0.2473	0.1917	0.6642**	0.1539
K			-0.5345**	0.2624	-0.2356	0.2789	-0.2615
Na				-0.1427	0.1818	-0.2624	0.1362
Ca					0.0581	0.3150	0.3803*
Mg						-0.416*0	0.2558
ALT							0.1811

\*, \*\* = Significant at P = 0.05 and 0.01, respectively

ALT = Aspartate Transaminase

URD = Ureide

Applied plant nutrients levels as mg P kg<sup>-1</sup> soil were 0, 100, 200, and 300 and mg K kg<sup>-1</sup> soil were 0 and 200.

Effects of Ca, P, and K factorial combination on growth, nodulation, and nitrogenase activity of winged bean are presented in Table IX.

The CaP treatment resulted in the significantly highest leaf dry matter of  $1.52 \text{ g plant}^{-1}$  followed by the combination of CaPK treatment of  $1.25 \text{ g plant}^{-1}$ . The lowest yield was obtained from P of  $0.55 \text{ g plant}^{-1}$ . The mean yield of the various treatment combinations was increased with P addition. When P was omitted the mean yield of Ca treatment was  $1.12 \text{ g plant}^{-1}$  and with P addition it was  $1.52 \text{ g plant}^{-1}$  as the significantly highest leaf dry weight. K affected positively the yields when added to the soil alone or when combined with Ca and CaP, but slightly depressed yield when combined with P only. The Ca effect was additive for treatments either with the single application or when combined with P, K, and PK. The level of significant difference at .05 was  $0.18 \text{ g plant}^{-1}$ .

The combination of CaPK resulted in the significantly highest stem dry weight with the value of  $1.06 \text{ g plant}^{-1}$  followed by the CaP treatment of  $1.02 \text{ g plant}^{-1}$  with 0.15 g as LSD at .05. The lowest yield was with the P treatment of  $0.48 \text{ g plant}^{-1}$ . The addition of Ca alone to the soil increased the yield from 0.53 to  $0.95 \text{ g plant}^{-1}$ . The addition of Ca to P, K, and CaP increased stem dry matter to 1.02, 0.93, and  $1.06 \text{ g plant}^{-1}$ , respectively. Therefore, Ca had positive effects for stem production. The P effect when combined with Ca, K, and CaK improved the yield with the following yields: CaP  $1.02 \text{ g}$ , KP  $0.77 \text{ g}$ , and CaPK  $1.06 \text{ g}$ , while the control was  $0.53 \text{ g}$ . The yield was depressed when P was applied alone with  $0.48 \text{ g}$  of stem dry weight. With the single application of K the yield was increased from  $0.53 \text{ g}$  to  $0.77 \text{ g}$ . When K was combined with Ca, P, and CaP the yields were increased from  $0.53 \text{ g}$  for the check to

TABLE IX  
EFFECTS OF Ca, P, AND K FACTORIAL COMBINATION ON GROWTH,  
NODULATION, AND NITROGENASE ACTIVITY OF WINGED  
BEAN GROWN ON A TYPIC EUTRUSTOX, SERIES I

Parameter	Treatment	0	P	K	PK	Ave
Leaf wt g.	0	0.57	0.55	0.63	0.62	0.59
	Ca	1.12	1.52	1.22	1.25	1.28
	Ave	0.85	1.04	0.92	0.93	(0.18)
Stem wt g.	0	0.53	0.48	0.77	0.77	0.64
	Ca	0.95	1.02	0.93	1.06	0.99
	Ave	0.74	0.75	0.85	0.92	(0.15)
Root wt g.	0	0.32	0.30	0.33	0.35	0.33
	Ca	0.72	0.63	0.57	0.47	0.66
	Ave	0.52	0.47	0.45	0.41	(0.10)
Nodule wt g.	0	0.18	0.40	0.25	0.44	0.32
	Ca	0.50	1.03	0.56	0.97	0.77
	Ave	0.34	0.72	0.41	0.71	(0.10)
Nodule No.	0	18	20	18	11	16.75
	Ca	34	63	37	39	43.25
	Ave	26	42	28	25	(9.43)
Nitrogenase	0	9.0	31.5	24.7	25.0	22.60
C <sub>2</sub> H <sub>4</sub> u mole	Ca	27.3	42.7	29.0	66.3	41.30
g <sup>-1</sup> nodule h <sup>-1</sup>	Ave	18.2	37.1	26.9	45.7	(6.32)

Plant nutrients additions as mg kg<sup>-1</sup> soil were 0, 1200 Ca, 200 P, and 200 K with analysis of variance as General Linear Model, LSD .05 in parenthesis of each parameter.

0.93, 0.77, and 1.06 g, respectively. There was no depressive effects with K additions to the soil.

The significantly highest yield for root growth was obtained by the addition of Ca that resulted in 0.72 g of dry matter compared to 0.32 g of the control yield. The lowest yield resulted with the application of P that slightly decreased the yield from 0.32 to 0.30 g plant<sup>-1</sup>. The value of 0.10 g for root dry weight was the level of significant difference at .05. Potassium effects were additive when applied alone or when combined with Ca, P, and CaP. The yields obtained were 0.33 when alone and 0.57, 0.35, and 0.47 g when combined with Ca, P, and CaP, respectively. Root growth was decreased when Ca was applied with P of 0.63 g, K of 0.57 g, and CaP of 0.47 g. However, the root yield was still higher than the check of 0.32 g. P combined with Ca, K, and CaK resulted in an increase of 0.63, 0.35, and 0.47 g of dry root matter, respectively against the control 0.32 g. The addition of P alone to the soil decreased the yield from 0.32 g to 0.30 g.

Significantly highest yield in fresh nodule weight was observed with the combination of P and Ca with a yield of 1.03 g plant<sup>-1</sup> and the lowest value was 0.18 g plant<sup>-1</sup> for the control with LSD .05 of 0.10 g. The single application of P to the soil resulted in higher yield than the control, 0.40 g for P. The P combination with Ca, K, and CaK increased yields resulting in 1.03 g for CaP, 0.44 g for PK, and 0.97 g for CaPK. The application of Ca either alone or combined resulted in increased yields when compared to the check, 0.18 g, with 0.50 g for Ca, 1.03 g for CaP, and 0.97 g for CaPK. The effect of K on fresh nodule weight was additive when alone or combined with Ca, P, and CaP. The single application of K increased the yield from 0.18 g for the control to 0.25

g, and when combined with Ca, P and CaP the yields were 0.44 g for PK, 0.56 g for CaP, and 0.97 g for CaPK.

The PCa treatment caused the significantly greatest nodule number of 63 nodules plant<sup>-1</sup> with the lowest of 11 nodules plant<sup>-1</sup> with PK treatment. The LSD .05 was 9.43 nodules plant<sup>-1</sup>. The addition of Ca alone increased nodule number from 18 nodules plant<sup>-1</sup> of the check to 34 nodules plant<sup>-1</sup>. With the combination of Ca to P, K, and PK, nodule number increased to 63, 37, and 39 nodules plant<sup>-1</sup>, respectively. These data indicated that Ca had an additive effect for nodule number. P increased nodule number from 18 nodules plant<sup>-1</sup> of the check to 20 nodules plant<sup>-1</sup> when applied alone and to 63 and 39 nodules plant<sup>-1</sup> when combined with Ca and CaK, respectively. However, number of nodule was not affected by P when applied with K yielding 18 nodules plant<sup>-1</sup>. The effect of K was depressive when applied with P with 11 nodules plant<sup>-1</sup> compared to the check of 18 nodules plant<sup>-1</sup>. Also, the depressive effect of K was apparent when combined with PCa because the number of nodules was decreased from 63 nodules plant<sup>-1</sup> as the highest nodule number to 39 nodules plant<sup>-1</sup>. However, the number of nodules was slightly increased from 34 nodules plant<sup>-1</sup> with Ca treatment to 37 nodules plant<sup>-1</sup> when K was applied with Ca. There was no effect on number of nodule when K was applied to the soil alone with 18 nodules plant<sup>-1</sup>.

The highest nitrogenase activity level was obtained with CaPK treatment with 66.3 C<sub>2</sub>H<sub>4</sub> u mole g<sup>-1</sup> nodule h<sup>-1</sup> and the lowest was with the check of 9 C<sub>2</sub>H<sub>4</sub> u mole g<sup>-1</sup> nodule h<sup>-1</sup>. The value of 6.32 C<sub>2</sub>H<sub>4</sub> u mole g<sup>-1</sup> nodule h<sup>-1</sup> was the LSD .05. P effect increased the nitrogenase activity from 9 for the control up to 31.5. When P combined with Ca, K, and CaK resulted in 25 of PK, 42.7 of CaP, and 66.3 of CaPK. The

addition of Ca resulted in nitrogenase activity 27.3 with the control 9. Ca effect when combined with P, K, and PK was also positive effect resulting in 42.7, 29, and 66.3, respectively. The effect of K addition to the soil resulted in increasing nitrogenase activity from 9 for the control to 24.7. The combination of K with Ca, P, and CaP resulted in CaK 29, KP 25, and CaPK 66.3  $\text{C}_2\text{H}_4$   $\mu\text{mole g}^{-1}$  nodule  $\text{h}^{-1}$ .

There were no plant nutrients applications with Series II determining residual responses following the Series I treatment. Table X shows the residual effects of P, K, and Ca combination on growth, nodulation, and nitrogenase activity level of winged bean on a Typic Eutruxox.

The significantly highest leaf dry weight was obtained with CaPK treatment, 1.20 g for dry matter, with the lowest obtained for K treatment yielding 0.27 g of leaf dry matter. The LSD .05 for leaf dry weight was 0.11 g. The residual effects of P application alone or combined with K, Ca, and CaK increased leaf growth yielding P 0.63 g, CaP 0.83 g, PK 0.70 g, and CaPK 1.20 g. K effect was depressive on the overall composite mean yield with single application of K the yield was 0.27 g compared to 0.40 g for the control. However, K in association with Ca, P, and CaP increased dry matter production, CaK 0.73 g, KP 0.70 g, and CaPK 1.20 g of dry matter. The leaf growth was not affected when Ca was applied alone to the soil in comparison to the control of 0.40 g. Ca effects when combined with P, K, and CaK were additive resulting values 0.83, 0.73, and 1.20 g, respectively.

The significantly highest yield 1.27 g stem dry matter with 0.15 g as LSD .05 was obtained when the soil was treated with CaPK combination followed by PK treatment resulting in 0.94 g yield. The lowest yield as



TABLE X  
EFFECTS OF RESIDUAL P, K, AND Ca COMBINATION ON GROWTH,  
NODULATION, AND NITROGENASE ACTIVITY OF WINGED BEAN  
GROWN ON A TYPIC EYTRYSTOX, SERIES II

Parameter	Treatment	0	P	K	PK	Ave
Leaf wt g.	0	0.40	0.63	0.27	0.70	0.50
	Ca	0.40	0.83	0.73	1.20	0.79
	Ave	0.40	0.73	0.50	0.95	(0.11)
Stem wt g.	0	0.53	0.50	0.56	0.94	0.63
	Ca	0.37	0.72	0.72	1.27	0.77
	Ave	0.45	0.23	0.47	0.39	(0.15)
Root wt g.	0	0.32	0.20	0.47	0.30	0.32
	Ca	0.34	0.23	0.47	0.39	0.40
	Ave	0.34	0.23	0.47	0.39	(0.07)
Nodule wt g.	0	0.35	0.98	0.49	0.85	0.66
	Ca	0.34	0.48	0.40	0.96	0.54
	Ave	0.34	0.73	0.44	0.90	(0.16)
Nodule No.	0	31	38	45	38	38.00
	Ca	15	18	29	43	26.25
	Ave	23	28	37	40	(11.65)
Nitrogenase	0	32.00	74.67	40.67	78.67	56.50
C <sub>2</sub> H <sub>4</sub> u mole	Ca	39.33	59.33	46.67	77.33	55.66
g <sup>-1</sup> nodule h <sup>-1</sup>	Ave	35.66	67.00	43.67	78.00	(7.02)

Residual of applied plant nutrients as mg kg<sup>-1</sup> soil were 0, 1200 Ca, 200 P, and 200 K with analysis of variance as General Linear Model, LSD .05 in parenthesis of each parameter.

the Ca treatment was 0.37 g. The addition of P to the soil decreased slightly the yield from 0.53 g for the control to 0.50 g dry matter. P combined with K, Ca, and CaK increased stem dry matter up to 0.94 g, 0.70, g and 1.27 g, respectively. Applying K to the soil alone or combined with Ca, P, and CaP increased yield. The control was 0.53 g with K 0.56 g, CaK 0.72 g, CaP 0.72 g, and CaPK 1.27 g of stem dry matter. Ca applied alone depressed the yield resulting in 0.37 g dry matter as the lowest yield. Positive effect of combining Ca with K, P, and PK was observed for CaK 0.72 g, CaP 0.72 g, and CaPK 1.27 g.

The significantly highest root dry matter was obtained with CaK treatment yielding 0.49 g dry matter compared to 0.32 g of the control. The lowest yield was from the P application with 0.20 g. The level of significant difference of root dry weight at .05 was 0.07 g. P when applied alone decreased yield from 0.32 g for the check to 0.20 g as the lowest yield. The combination of P with Ca also depressed root growth with 0.27 g of root dry matter in comparing to the check. However, root growth was increased when P was combined with both Ca and K as CaPK treatment of 0.49 g root dry matter. Applying K alone and combination with Ca and PCa increased root growth with 0.47, 0.48, and 0.49 g, respectively. The combination of K with P slightly depressed root dry weight from 0.32 g of the control to 0.30 g as PK treatment. Root growth was increased when Ca was applied alone and combination with P, K, and PK as 0.37 g of Ca, 0.48 g of CaP, and 0.49 g of CaPK with 0.32 g of the control.

The significantly largest nodule fresh weight was obtained by treating the soil with P treatment 0.98 g and the lowest yield was obtained with Ca applied alone 0.34 g with the LSD .05 of 0.16 g.

Treating soil with P increased nodule fresh weight from 0.35 g for the control up to 0.98 g. Comparing the effects of combining P with Ca, K, and CaK yield results were 0.48, 0.85, and 0.96 g, respectively. Ca as a single treatment slightly depressed yield with the control yield 0.35 g and Ca treatment 0.34 g. When Ca was combined with P, K, and PK yields were increased as CaK 0.40 g, CaP 0.48 g, and CaPK 0.96 g. Applying K to soil as a single treatment increased yield 0.49 g and increase in yield also occurred when K was combined with P, Ca, and PCa, 0.85 g, 0.40 g, and 0.96 g, respectively.

The significantly greatest nodule number was obtained with K treatment, 45 nodules plant<sup>-1</sup> and the lowest nodule number was obtained when soil was treated with single application of Ca, 15 nodules plant<sup>-1</sup> with LSD .05 of 11.6 nodules plant<sup>-1</sup>. The combination application of P with Ca depressed the yield from 31 nodules for the control to 18 nodules. The single application of P with K and CaK increased nodule development yielding 38 nodules of P, 38 nodules of PK, and 43 nodules of PKCa treatment. Ca applied alone decreased nodule development to 15 nodules in comparing to the check of 31 nodules. Although, Ca was combined with K and P decreased nodule number with 18 for CaP and 29 for CaK. However, the nodule number was increased when Ca was combined with both K and P with 43 nodules plant<sup>-1</sup> in comparison to the control of 31 nodules plant<sup>-1</sup>.

Nitrogenase activity was significantly highest when soil was treated with PK resulting in 78.67 u mole with 7.02 u mole C<sub>2</sub>H<sub>4</sub> as LSD .05. The lowest was obtained from the check of 32 u moles. The addition of P resulted in higher nitrogenase activity of 74.67 with the control 32 u moles. P effect when combined with Ca, K, and CaK was also positive

resulting in values 59.33 of CaP, 78.67 of PK, and 77.33 of CaPK.

Treating soil with K increased nitrogenase activity up to 40.67 with the control of 32 u moles. When K was combined with Ca, P, and CaP resulted in 78.67 for PK, 46.67 for CaK, and 77.33 for CaPK treatment. The effect of adding Ca to the soil resulted in increasing nitrogenase activity from 32 u moles for the control to 39.33 u moles. The combination of Ca with K, P, and PK resulted in KCa 46.67, PCa 59.33, and PKCa 77.33 u moles.

Correlation coefficients of measured parameters as influenced by soil treatments of Ca, P, and K factorial combination for Series I ( fertilized ) and Series II ( residual ) are shown in Table XI.

Significant correlation coefficients were shown in Series I for nitrogenase X leaf (0.488\*), and nitrogenase X nodule weight (0.792\*\*). Also, correlation coefficients were obtained for nodule fresh weight X stem (0.681\*\*), nodule fresh weight X leaf (0.787\*\*), nodule fresh weight X root (0.535\*) and nodule fresh weight X number of nodule (0.732\*\*); nodule number X stem (0.794\*\*), nodule number X leaf (0.897\*\*) and nodule number X root (0.709\*\*); root X stem (0.707\*\*), and root X leaf (0.729\*\*); and leaf X stem (0.857\*\*).

Significant correlation coefficients were apparent for residual effects in Series II for nitrogenase X leaf (0.434\*), nitrogenase X nodule fresh weight (0.481\*), nodule fresh weight X leaf (0.529\*), nodule fresh weight X stem (0.564\*), nodule fresh weight X nodule number (0.545\*), root X leaf (0.567\*), and stem X leaf (0.830\*\*).

Effects of Ca, P, and K factorial combination on nodule cytosol composition of P, K, Na, Ca, Mg, aspartate transaminase, and ureide are presented in Table XII.

The significantly highest P cytosol of 528 P ug/g of nodule

TABLE XI

CORRELATION COEFFICIENTS OF PLANT GROWTH PARAMETERS, NITROGENASE,  
AND NODULATION OF WINGED BEAN AS AFFECTED BY  
Ca, P, AND K FACTORIAL COMBINATION

Parameter	Series I					
	Stem	Leaf	Root	Nod No	Nod Wt	Nase
Stem		0.8576**	0.7074**	0.7946**	0.6812**	0.3955
Leaf	0.8306**		0.7295**	0.8976**	0.7874**	0.4887*
Root	0.5674*	0.3199		0.7091**	0.5354*	0.2103
Nod No	0.3208	0.1532	0.3276		0.7322**	0.3581
Nod Wt	0.5640*	0.5294*	0.1740	0.5459*		0.7923**
Nase	0.3007	0.4343*	-0.2426	0.2855	0.4811*	
Series II						

\*, \*\* = Significant at P = 0.05 and 0.01, respectively  
Plant nutrients applied for Series I  
Residual response of applied nutrients for Series II

TABLE XII  
EFFECTS OF Ca, P, AND K COMBINATIONS ON COMPOSITION OF NODULE  
CYTOSOL OF WINGED BEAN GROWN ON A TYPIC EYTRUSTOX, SERIES I

Parameter	Treatment	0	P	K	PK	Ave
P ug/g	0	188	448	144	284	276.00
	Ca	528	220	272	260	320.00
	Ave	358	354	208	272	(12.2)
K ug/g	0	1715	2325	3440	1375	2213.75
	Ca	3540	1780	3150	2875	2836.25
	Ave	2628	2026	3295	2125	(125.5)
Na ug/g	0	180	230	190	225	206.25
	Ca	120	175	150	130	143.75
	Ave	150	203	170	178	(10.1)
Ca ug/g	0	59	72	78	99	76.87
	Ca	111	78	62	78	82.12
	Ave	85	75	70	89	(13.1)
Mg ug/g	0	210	88	60	80	109.37
	Ca	165	80	100	100	111.25
	Ave	188	84	80	90	(11.1)
Aspartate	0	13.5	83.5	17.0	12.5	31.62
Transaminase	Ca	78.0	48.5	13.5	82.5	55.62
IU/g	Ave	45.7	66.0	15.2	47.5	(5.5)
Ureide u mole/g	0	67.0	47.0	57.5	83.5	63.75
	Ca	72.0	47.0	47.5	57.5	56.00
	Ave	69.5	47.0	52.5	70.5	(6.0)

Plant nutrients additions as mg kg<sup>-1</sup> soil were 0, 1200 Ca, 200 P, and 200 K with analysis of variance as General Linear Model, LSD .05 in parenthesis of each parameter.

resulted with added Ca to the soil, the lowest cytosol P was obtained when the soil was not treated with any plant nutrients, 188 P ug/g nodule. The .05 level of significance was 12.2 ug/g nodule. Single application of P increased P uptake from 188 ug/g for the control to 448 ug/g nodule. However, the combination of P with Ca, K, and CaK increased cytosol P as 220 of PCa, 284 of PK, and 260 of PKCa treatment. The effect of Ca treatments increased cytosol P either alone or combined. For Ca alone the result was 528 ug/g nodule, when combined with P 220 ug/g, K 272, and PK 260 ug/g. Applying K alone to the soil reduced P cytosol content, 144 ug/g, but K combined with Ca, P, and CaP increased P cytosol to 272, 284, and 260 ug/g, respectively.

The significantly highest cytosol K was obtained with Ca treatment, 3540 ug/g, the lowest cytosol K was apparent from the check, 1715 ug/g with LSD .05 of 125.5 ug K/g. Ca effect increased cytosol K from 1715 ug/g for the control up to 3540 ug/g. When the Ca effect combined with K, P, and KP resulted with CaP 1780 ug/g, CaK 3150 ug/g, and CaPK 2875 ug/g. The addition of K to the soil resulted in higher K cytosol of 3440 ug/g with the control of 1715 ug/g. K effect when combined with P, Ca, and PCa was also positive with resulting values of 1375, 3150, and 2785 ug/g, respectively. The influence of applying P to the soil was beneficial since the cytosol K was increased from 1715 for the control to 2325 ug/g. P combined with K, Ca, and CaK resulted in the following yields 1375, 1780, and 2875 ug/g, respectively.

The treatment that resulted in the significantly highest cytosol Na content, 230 ug/g was from P treatment and the lowest 120 ug/g was obtained from Ca treatment with 10.1 ug/g nodule as LSD .05. The single application of P to the soil increased Na uptake from 180 ug/g to 230

ug/g. When P combined with K also enhanced Na cytosol content with 225 ug/g. However, the effect of P in the presence of Ca and CaP decreased Na cytosol content with 175 and 130 ug/g, respectively. When K alone was applied to the soil Na content raised from 180 ug/g to 190 ug/g. The effect of K combined with P also improved Na content as 225 ug/g of PK treatment. When K was added with Ca and CaP resulted in lower Na content as 150 for KCa and 130 for PKCa.

The significantly highest Ca cytosol content 111 ug/g was observed from the cultures treated with Ca treatment. The lowest Ca content was due to the control, 59 ug/g. The level of significant difference at .05 was 13.1 ug Ca/g nodule. The single application of Ca to the soil was positive resulting in higher Ca content than the control, 111 for Ca. The Ca combination with P, K, and PK increased Ca cytosol content as 78 ug/g for CaP, 62 ug/g for CaK, and 78 ug/g for CaPK. The application of P either alone or combined resulted in increased Ca cytosol content when compared to the control 59 ug/g, with 72 ug/g of P, 78 ug/g for CaP, 99 ug/g for PK, and 78 ug/g for CaPK. When K was added to the soil Ca content was increased from 59 ug/g for the control up to 78 ug/g. The K effect in combination with P, Ca, and CaP also increased Ca content in the cytosol with 99 ug/g for PK, 62 for CaK, and 78 ug/g for CaPK.

The control resulted in significantly highest Mg cytosol content of 210 ug/g, the lowest Mg cytosol was obtained from K treatment of 60 ug/g with the LSD .05 of 11.1 ug/g nodule. P effect decreased Mg cytosol from 210 ug/g for the control to 88 ug/g. Mg cytosol was also decreased when P was combined with K, Ca, and CaK resulting in 80 ug/g for PK, 80 ug/g for PCa, and 100 ug/g for PKCa. The addition of K resulted in



lowest Mg content of 60 ug/g with the control of 210 ug/g. K effect when combined with P, Ca, and PCa was also negative effect resulting in 80 ug/g, 100 ug/g, and 100 ug/g, respectively. Treating soil with Ca decreased Mg cytosol from 210 ug/g of the control to 165 ug/g. When Ca was combined with P, K, and PK resulted in 80 ug/g, 100 ug/g, and 100 ug/g for CaP, CaK, and PKCa treatment, respectively.

The significantly highest aspartate transaminase was obtained with P treatment of 83.5 IU/g with the lowest of 13.5 IU/g from the control. The .05 significant level was 5.50 IU/g. Single applied P to the soil increased aspartate transaminase from 13.5 IU/g for the control to 83.5 IU/g. The effect of P in combination with other elements was beneficial in all treatments except with K. For the combination CaP 48.5 IU/g was obtained, and for PK and CaPK 12.5 and 82.5 IU/g were obtained, respectively. When K was applied alone the response was 17 IU/g which was higher than the control, 13.5 IU/g. The application of K with Ca resulted in 13.5 IU/g, K combined with P produced 12.5 IU/g which was lower than the control while PKCa was 82.5 IU/g. With single application of Ca, aspartate transaminase was 78 IU/g compared to 13.5 for the control. Also, Ca in association with P and PK increased aspartate transaminase activity as PCa 48.5 IU/g, PKCa 82.5 IU/g. However, the combination of Ca with K did not effect aspartate transaminase in comparing with the check, 13.5 IU/g.

The significantly highest ureide content of 83.5 u moles/g was obtained when the soil was treated with PK treatment. The lowest ureide as P and PCa treatments resulted in 47 u moles/g. The .05 level of significance was 6.0 u moles/g. The effect of applying P alone or combined with Ca and CaK decreased ureide content. The control had

ureide content of 67 u moles/g but ureide content of P, PCa, and PKCa were 47, 47.5, and 57.5 u moles/g, respectively. However, PK treatment increased ureide content up to 83.5 u moles/g over the check of 67 u moles/g. K when applied with P increased ureide content from 67 u moles/g for the control to 83.5 u moles/g. K alone and combination with Ca, and CaP depressed ureide content as 57.5 for K, 47.5 for CaK, and 57.5 for CaPK treatment with the control of 67 u moles/g. Ca as a single treatment increased ureide content with the control 67 u moles/g and Ca treatment 72 u moles/g. All Ca combination with K, P, and PK depressed ureide content as 47.5, 47, and 57.5 u moles/g, respectively.

The effects of four K levels with and without P combination on growth, nodulation, and nitrogenase activity of winged bean os Series I are shown in Table XIII.

Increased K levels increased leaf dry weight as quadratic with the significantly highest leaf dry weight of  $1.23 \text{ g plant}^{-1}$  of  $PK_3$  treatment and the lowest leaf dry weight of  $0.52 \text{ g plant}^{-1}$  of  $PK_2$  treatment with LSD .05 of  $0.32 \text{ g}$ . P combined with K levels caused lower leaf dry weight as  $0.89 \text{ g plant}^{-1}$  for pooled  $P_1$  and  $1.06 \text{ g plant}^{-1}$  for pooled  $P_0$ .

The stem dry weight increased as quadratic response with the increased pooled K levels and increased K levels. With P addition caused lower stem dry weight from  $1.11 \text{ g plant}^{-1}$  of pooled  $P_0$  to  $0.85 \text{ g plant}^{-1}$  of pooled  $P_1$ . The highest  $K_1$  treatment as  $1.43 \text{ g plant}^{-1}$  was significantly higher stem dry weight than the lowest  $PK_2$  treatment of  $0.64 \text{ g plant}^{-1}$  with  $0.31 \text{ g}$  as LSD .05.

The significantly highest root dry weight was  $0.43 \text{ g plant}^{-1}$  of  $K_1$  treatment as compared to the lowest  $0.23 \text{ g plant}^{-1}$  of  $PK_2$  treatment. The level of significant difference at .05 was  $0.08 \text{ g}$ . A quadratic response was apparent with increased K levels and pooled K levels. There was a slight change from P effect of  $0.32 \text{ g plant}^{-1}$  as pooled  $P_1$  compared to pooled  $P_0$  of  $0.38 \text{ g plant}^{-1}$ .

Fresh nodule weight increased as quadratic response with increasing K levels and pooled K levels. P effect decreased fresh nodule weight from  $0.95 \text{ g plant}^{-1}$  as pooled  $P_0$  to  $0.65 \text{ g plant}^{-1}$  as pooled  $P_1$ . The  $K_2$  treatment gave the significantly highest fresh nodule weight of  $1.20 \text{ g plant}^{-1}$  with the lowest of  $0.59 \text{ g per plant}$  for  $PK_3$  treatment with  $0.20 \text{ g}$  as LSD .05.

TABLE XIII  
EFFECTS OF K LEVELS WITH AND WITHOUT P COMBINATION ON GROWTH,  
NODULATION, AND NITROGENASE ACTIVITY OF WINGED BEAN  
GROWN ON A TYPIC EUTRUSTOX, SERIES I

Parameter	Treatment	0	K <sub>1</sub>	K <sub>2</sub>	K <sub>3</sub>	Ave
Leaf wt g.	0	1.10	1.20	1.20	0.72	1.06
	P	1.00	0.75	0.52	1.23	0.89
	Ave	1.05	0.98	0.88	0.98	(0.32)
Stem wt g.	0	1.12	1.43	1.17	0.70	1.11
	P	1.03	0.75	0.64	0.98	0.85
	Ave	1.08	1.09	0.91	0.84	(0.31)
Root wt g.	0	0.35	0.43	0.40	0.35	0.38
	P	0.38	0.42	0.23	0.25	0.32
	Ave	0.37	0.43	0.32	0.30	(0.08)
Nodule wt g.	0	0.84	1.03	1.20	0.74	0.95
	P	0.69	0.66	0.67	0.59	0.65
	Ave	0.77	0.85	0.94	0.67	(0.20)
Nodule No.	0	36	29	19	33	29
	P	20	16	14	25	19
	Ave	28	22	17	29	(8.10)
Nitrogenase C <sub>2</sub> H <sub>4</sub> u mole g <sup>-1</sup> nodule h <sup>-1</sup>	0	88.3	176.0	188.6	212.0	166.20
	P	88.6	141.3	144.0	207.0	145.20
	Ave	88.5	158.6	166.3	209.5	(28.8)

Applied plant nutrients levels as mg K kg<sup>-1</sup> soil were 0, 200, 400, and 600 and mg P kg<sup>-1</sup> soil were 0, and 200 with analysis of variance as General Linear Model, LSD .05 in parenthesis of each parameter.

Number of nodule decreased due to effects of K levels with P addition with pooled  $P_0$  19 nodules plant<sup>-1</sup> compared to pooled  $P_1$ , 29 nodules plant<sup>-1</sup>. However, the check showed the significantly highest nodule number per plant of 36 nodules plant<sup>-1</sup> with the lowest of 14 nodules plant<sup>-1</sup> with  $PK_2$  treatment. The LSD .05 was 8.10 nodules plant<sup>-1</sup>.

The highest  $K_3$  treatment with 212  $C_2H_4$  u mole g<sup>-1</sup> nodule h<sup>-1</sup> was significantly higher in nitrogenase activity level than the lowest 88.3  $C_2H_4$  u mole g<sup>-1</sup> nodule h<sup>-1</sup> from the control. The LSD .05 was 28.8  $C_2H_4$  u mole g<sup>-1</sup> nodule h<sup>-1</sup>. K levels linearly increased nitrogenase activity from 88.3  $C_2H_4$  u mole g<sup>-1</sup> nodule h<sup>-1</sup> of  $K_0$  treatment to 212  $C_2H_4$  u mole g<sup>-1</sup> nodule h<sup>-1</sup> of  $K_3$  treatment. However, nitrogenase activity was slightly decreased with the effect of P addition from 166.2  $C_2H_4$  u mole g<sup>-1</sup> nodule h<sup>-1</sup> for pooled  $P_0$  to 145.2  $C_2H_4$  u mole g<sup>-1</sup> nodule h<sup>-1</sup> for pooled  $P_1$ .

Residual effects of K levels with and without P combination on growth, nodulation, and nitrogenase activity of winged bean on a Typic Eutruxox are shown on Table XIV.

Leaf dry weight yielded a quadratic response with increased K levels and increased pooled K levels. There was no effect with P addition of 0.84 g plant<sup>-1</sup>. The  $PK_1$  treatment had the significantly highest leaf dry matter with 1.18 g plant<sup>-1</sup> as compared to the lowest 0.67 g plant<sup>-1</sup> from the check. The LSD .05 was 0.01 g plant<sup>-1</sup>.

The effect of Pooled P addition increased stem dry weight from 0.77 g plant<sup>-1</sup> for  $P_0$  to 0.80 g plant<sup>-1</sup> of  $P_1$ . Pooled K levels increased stem dry weight in quadratic response with highest of 1.06 g plant<sup>-1</sup> compared to  $K_0$  of 0.60 g plant<sup>-1</sup>. The significantly highest stem dry

TABLE XIV  
EFFECTS OF RESIDUAL K LEVELS WITH AND WITHOUT P COMBINATION  
ON GROWTH, NODULATION, AND NITROGENASE ACTIVITY OF WINGED  
BEAN GROWN ON A TYPIC EUTRUSTOX, SERIES II

Parameter	Treatment	0	K <sub>1</sub>	K <sub>2</sub>	K <sub>3</sub>	Ave
Leaf wt g.	0	0.67	1.05	0.76	0.86	0.84
	P	0.80	1.18	0.81	0.58	0.84
	Ave	0.74	1.12	0.79	0.72	(0.10)
Stem wt g.	0	0.50	0.93	0.76	0.88	0.77
	P	0.70	1.19	0.86	0.46	0.80
	Ave	0.60	1.06	0.81	0.67	(0.02)
Root wt g.	0	0.27	0.27	0.36	0.27	0.29
	P	0.37	0.41	0.36	0.25	0.35
	Ave	0.32	0.34	0.36	0.26	(0.07)
Nodule wt g.	0	0.46	0.63	0.57	0.55	0.55
	P	0.47	0.74	0.61	0.35	0.54
	Ave	0.46	0.68	0.59	0.45	(0.12)
Nodule No	0	16	16	18	22	18
	P	14	46	33	16	27
	Ave	15	31	25	19	(10.24)
Nitrogenase C <sub>2</sub> H <sub>4</sub> u mole g <sup>-1</sup> nodule h <sup>-1</sup>	0	18.33	31.00	67.00	41.33	39.41
	P	32.67	32.33	27.67	25.00	29.41
	Ave	25.50	31.66	47.33	33.16	(8.37)

Residual of applied plant nutrients levels as mg K kg<sup>-1</sup> soil were 0, 200, 400, and 600 and P kg<sup>-1</sup> soil were 0, and 200 with analysis of variance as General Linear Model, LSD .05 in parenthesis of each parameter.

matter resulted from PK<sub>1</sub> treatment of 1.19 g plant<sup>-1</sup> with LSD .05 of 0.02 g and the lowest stem weight was obtained from PK<sub>3</sub> treatment of 0.46 g plant<sup>-1</sup>.

Quadratic increase in root growth occurred from increased pooled K levels with the highest of K<sub>2</sub> of 0.36 g plant<sup>-1</sup> compared to K<sub>0</sub> of 0.32 g plant<sup>-1</sup>. Pooled P addition increased root growth from P<sub>0</sub>, 0.29 g plant<sup>-1</sup> to P<sub>1</sub>, 0.35 g plant<sup>-1</sup>. The lowest PK<sub>3</sub> treatment was 0.25 g plant<sup>-1</sup> and the highest PK<sub>1</sub> treatment was 0.41 g plant<sup>-1</sup>. The level of significant difference at .05 was 0.07 g.

Comparison of the highest fresh nodule weight of PK<sub>1</sub> treatment with 0.74 g plant<sup>-1</sup> was significantly higher than the lowest from PK<sub>3</sub> treatment of 0.35 g plant<sup>-1</sup> with LSD .05 of 0.12 g. A quadratic response was apparent with increased pooled K levels with highest of K<sub>1</sub> 0.68 g plant<sup>-1</sup>. The addition of Pooled P effects slightly decreased fresh nodule weight from 0.55 g plant<sup>-1</sup> for P<sub>0</sub> to 0.54 g plant<sup>-1</sup> for P<sub>1</sub>.

Increased pooled K levels resulted in a quadratic increase in number of nodules with highest K<sub>1</sub> of 31 nodules plant<sup>-1</sup> and the lowest K<sub>0</sub> of 15 nodules plant<sup>-1</sup>. P also caused the increase in nodule number from 18 nodules plant<sup>-1</sup> of P<sub>0</sub> to 27 nodules plant<sup>-1</sup> of P<sub>1</sub>. The highest PK<sub>1</sub> treatment 46 nodules plant<sup>-1</sup> was significantly greater than the lowest PK<sub>3</sub>, K<sub>1</sub>, and the control with 16 nodules per plant. The LSD .05 was 10.24 nodules plant<sup>-1</sup>.

Pooled K levels showed an increase in nitrogenase activity in quadratic response with the highest of K<sub>2</sub> 47.33 C<sub>2</sub>H<sub>4</sub> u mole g<sup>-1</sup> nodule h<sup>-1</sup> and the lowest of K<sub>0</sub> 25.50 C<sub>2</sub>H<sub>4</sub> u mole g<sup>-1</sup> nodule h<sup>-1</sup>. Nitrogenase activity was decreased with the addition of P from 39.41 C<sub>2</sub>H<sub>4</sub> u mole g<sup>-1</sup> nodule h<sup>-1</sup> of P<sub>0</sub> down to 29.41 C<sub>2</sub>H<sub>4</sub> u mole g<sup>-1</sup> nodule h<sup>-1</sup> of P<sub>1</sub>. The

highest  $\text{PK}_2$  treatment with  $67 \text{ C}_2\text{H}_4 \text{ u mole g}^{-1} \text{ nodule h}^{-1}$  was significantly higher than the lowest  $18.33 \text{ C}_2\text{H}_4 \text{ u mole g}^{-1} \text{ nodule h}^{-1}$  for the control. The LSD .05 was  $8.37 \text{ C}_2\text{H}_4 \text{ u mole g}^{-1} \text{ nodule h}^{-1}$ .

Correlation coefficients of growth parameters as influenced by K levels with and without P addition for Series I ( fertilized ) and Series II ( residual ) are presented in Table XV.

Significant correlation coefficients of Series I were nitrogenase X fresh nodule weight ( $0.500^*$ ), fresh nodule weight X stem ( $0.600^{**}$ ), fresh nodule weight X root ( $0.486^*$ ), fresh nodule weight X number of nodule ( $0.477^*$ ), number of nodule X stem ( $0.482^*$ ), root X stem ( $0.631^{**}$ ), root X leaf ( $0.525^*$ ), and stem X leaf ( $0.897^{**}$ ).

In Series II the significant correlation coefficients of fresh nodule weight were with stem ( $0.869^{**}$ ), leaf ( $0.853^{**}$ ), root ( $0.473^*$ ), and number of nodule ( $0.659^{**}$ ). Number of nodules showed significant correlation coefficients with stem ( $0.585^*$ ) and leaf ( $0.592^*$ ). Root was significantly correlated with stem ( $0.496^*$ ) and leaf ( $0.521^*$ ). The significant correlation coefficient of stem and leaf was  $0.920^{**}$ .



TABLE XV

CORRELATION COEFFICIENTS OF PLANT GROWTH PARAMETERS,  
NITROGENASE, AND NODULATION OF WINGED BEAN AS  
AFFECTED BY K LEVELS WITH AND WITHOUT P

Series I						
Parameter	Stem	Leaf	Root	Nod No	Nod wt	Nase
Stem		0.8973**	0.6311**	0.4822*	0.6004**	0.3121
Leaf	0.9204**		0.5258*	0.4482*	0.6158**	0.3871
Root	0.4968*	0.5214*		0.2589	0.4860*	0.2389
Nod No	0.5859*	0.5927*	0.3540		0.4777*	0.3547
Nod wt	0.8690**	0.8534**	0.4737*	0.6597**		0.5002*
Nase	0.2515	0.1662	0.2741	0.0172	0.2550	
Series II						

\*, \*\* = Significant at P = 0.05 and 0.01, respectively  
Plant nutrients applied for Series I  
Residual response of applied nutrients for Series II

The effects of P, K, and trace element combination on growth, nodulation, and nitrogenase activity of winged bean of Series I are shown in Table XVI.

The significantly highest leaf dry weight of  $1.62 \text{ g plant}^{-1}$  was obtained by Ptr treatment and the lowest leaf dry weight was from P treatment of  $0.78 \text{ g plant}^{-1}$  with LSD .05 of  $0.17 \text{ g}$ . Application of P alone decreased leaf growth from  $0.82 \text{ g}$  for the check to  $0.78 \text{ g}$ . When P combined with K, tr, and Ktr the yield was increased to  $1.28$ ,  $1.62$ , and  $1.17 \text{ g}$ , respectively. With the application of K alone or combination with P, tr, and Ptr, there was a significant increase in leaf growth as  $1.22$  for K,  $1.25$  for Ktr, and  $1.17$  for PKtr. The trace elements influenced positively leaf dry weight yielding  $1.45 \text{ g}$  when applied alone against  $0.82 \text{ g}$  for the control. All trace elements yields had higher leaf dry weight than the control and the effects of applying trace elements combined with P, K, and PK were  $1.62 \text{ g}$  for Ptr,  $1.25 \text{ g}$  for Ktr, and  $1.17 \text{ g}$  for PKtr.

Stem dry weight was significantly highest with PK treatment of  $1.70 \text{ g plant}^{-1}$  and the lowest was with P treatment of  $0.55 \text{ g plant}^{-1}$  with  $0.28 \text{ g}$  as LSD .05. The addition of P alone depressed stem growth resulting in the lowest stem growth,  $0.53 \text{ g}$  with the control of  $0.55 \text{ g}$ . When P combined with K, tr, and Ktr resulted in  $1.70 \text{ g}$  for PK,  $1.38 \text{ g}$  for Ptr, and  $1.53 \text{ g}$  for PKtr. The influence of applying K to the soil was beneficial as indicated by the increased yield from  $0.55 \text{ g}$  for the control to  $1.23 \text{ g}$ . When K combined with P, tr, and Ptr the yield was obtained as  $1.70 \text{ g}$ ,  $1.43 \text{ g}$ , and  $1.53 \text{ g}$ , respectively. The effects of trace elements alone or combined with P, K, and PK were tr  $1.50 \text{ g}$ , Ptr  $1.38 \text{ g}$ , Ktr  $1.43 \text{ g}$ , and PKtr  $1.53 \text{ g}$  with the control  $0.55 \text{ g}$ .

TABLE XVI  
EFFECTS OF P, K, AND TRACE ELEMENT COMBINATION ON GROWTH,  
NODULATION, AND NITROGENASE ACTIVITY OF WINGED BEAN  
GROWN ON A TYPIC EUTRUSTOX, SERIES I

Parameter	Treatment	0	P	tr	Ptr	Ave
Leaf wt g.	0	0.82	0.78	1.45	1.62	1.17
	K	1.22	1.28	1.25	1.17	1.23
	Ave	1.02	1.03	1.35	1.40	(0.17)
Stem wt g.	0	0.55	0.53	1.50	1.38	0.99
	K	1.23	1.70	1.43	1.53	1.47
	Ave	0.89	1.12	1.47	1.46	(0.28)
Root wt g.	0	0.33	0.25	0.53	0.48	0.40
	K	1.08	1.29	0.84	1.35	1.14
	Ave	0.82	0.84	0.99	1.28	(0.18)
Nodule No	0	14	15	33	32	23.50
	K	61	78	29	64	58.00
	Ave	37.5	46.5	48.5	48.0	(7.85)
Nodule wt g.	0	0.55	0.39	1.13	1.21	0.82
	K	1.08	1.29	0.84	1.35	1.14
	Ave	0.82	0.84	0.99	1.28	(0.18)
Nitrogenase C <sub>2</sub> H <sub>4</sub> u mole g <sup>-1</sup> nodule h <sup>-1</sup>	0	36.3	29.0	58.3	48.3	43.00
	K	38.6	36.0	33.6	42.0	37.59
	Ave	37.5	32.5	46.0	45.1	(7.85)

Applied plant nutrient levels as mg kg<sup>-1</sup> soil were 0, 100 P, 200 K, and 200 tr with analysis of variance as General Linear Model, LSD .05 in parenthesis of each parameter.

The significantly highest root dry weight resulted with the PKtr treatment, 1.35 g, with the smallest of 0.25 g from P treatment. The .05 level of significant difference was 0.18 g. P effect was depressive on root growth when applied alone. With single application of P the yield was 0.25 g compared to 0.33 g for the control. P in association with K, tr, and Ktr increased dry root production, PK 1.29 g, Ptr 0.48 g, and PKtr 1.35 g of dry matter. K applied to the soil either alone or combination with P, tr, and Ptr increased yield. The control yield was 0.33 g, K 1.08 g, Ktr 0.84 g, and PKtr 1.35 g root dry matter. Applying trace elements alone or combined increased yield. The control was 0.33 g with tr 0.84 g, Ptr 0.48 g, Ktr 0.84 g, and PKtr 1.35 g.

The largest number of nodules was obtained with the application of PK resulting in 78 nodules plant<sup>-1</sup>, the lowest nodule number was 14 from the control with LSD .05 of 7.85 nodules plant<sup>-1</sup>. The effect of adding P to the soil slightly increased nodule number from 14 for the control to 15 nodules plant<sup>-1</sup>. When P was added with K, tr, and Ktr the results were 78, 32 and 64 nodules plant<sup>-1</sup>, respectively. Single application of K resulted in 61 nodules plant<sup>-1</sup>. When this element was combined with P, tr and Ptr the results were still positive with 78, 29, and 64 nodules plant<sup>-1</sup>, respectively. The application of trace elements alone or combination resulted in increased nodule number per plant when compared to the control 14 nodules plant<sup>-1</sup>, with 33 nodules for tr, 32 nodules for Ptr, 29 nodules for Ktr, and 64 nodules for PKtr.

Significantly highest fresh nodule weight was 1.35 g from PKtr treatment with LSD .05 of 0.18 g. The lowest fresh nodule weight was obtained from P addition alone, 0.39 g. Results with P addition indicated a depressive effect to fresh nodule weight, 0.39 g compared to

the control of 0.55 g. The fresh nodule weight was increased when P was combined with K, tr and Ktr with 1.29, 1.21 and 1.35 g respectively. K effected positively to the fresh nodule weight either alone or combination with K, tr and Ktr. For the combination KP 1.29 g was obtained and for Ktr and PKtr 0.84 and 1.35 g were obtained. The effect of applying trace elements was positive in all treatments. Fresh nodule weight was 1.13 with single trace elements application with the control, 0.55. Trace elements combined with P, K and PK produced 1.21, 0.84, and 1.35 g of fresh nodule weight, respectively.

The significantly highest nitrogenase activity was  $58.3 \text{ C}_2\text{H}_4 \text{ u mole g}^{-1} \text{ nodule h}^{-1}$  with tr treatment and the lowest was  $29 \text{ C}_2\text{H}_4 \text{ u mole g}^{-1} \text{ nodule h}^{-1}$  from P treatment with LSD .05 as 7.85. P effect decreased nitrogenase activity from 36.3 for the control to 29. Slightly decreased nitrogenase activity was caused by PK treatment. However, nitrogenase activity was increased when P was combined with Ktr as PKtr treatment, 42. The addition of K resulted in higher nitrogenase activity with 38.6, the control 36.3. The combination of K with P and tr reduced the nitrogenase activity down to 36 and 33.6. However, nitrogenase activity was raised when K was combined with Ptr, 42. When the soil received trace elements alone or in combination increased nitrogenase activity except with K. The nitrogenase activity of tr, Ptr, and PKtr were 58.33, 48.33 and 42, respectively. The combination of tr with K depressed nitrogenase activity down to 33.6.

Table XVII presents the residual effects of P, K, and tr combination on growth, nodulation, and nitrogenase activity level of winged bean grown on a Typic Eutruxox.

The treatment that resulted in the significantly highest leaf

TABLE XVII  
EFFECTS OF RESIDUAL P, K, AND TRACE ELEMENT COMBINATIONS ON  
GROWTH, NODULATION, AND NITROGENASE ACTIVITY OF WINGED  
BEAN GROWN ON A TYPIC EUTRUSTOX, SERIES II

Parameter	Treatment	0	P	tr	Ptr	Ave
Leaf wt g.	0	0.93	0.83	0.62	0.72	0.78
	K	0.83	1.01	0.93	1.40	1.04
	Ave	0.88	0.92	0.78	1.06	(0.21)
Stem wt g.	0	0.53	0.52	0.40	0.46	0.48
	K	0.88	1.02	0.94	1.87	1.18
	Ave	0.70	0.77	0.67	1.66	(0.19)
Root wt g.	0	0.42	0.32	0.26	0.33	0.33
	K	0.53	0.56	0.52	0.63	0.56
	Ave	0.48	0.44	0.39	0.48	(0.09)
Nodule wt g.	0	0.47	0.49	0.29	0.31	0.38
	K	0.55	0.63	0.58	1.19	0.74
	Ave	0.51	0.56	0.42	0.75	(0.14)
Nodule No	0	20	20	18	9	16.75
	K	47	35	56	49	46.75
	Ave	33.5	27.5	37.0	29.0	(11.67)
Nitrogenase	0	31.0	49.6	22.0	46.5	37.29
C <sub>2</sub> H <sub>4</sub> u mole	K	33.6	42.3	40.6	47.3	41.00
g <sup>-1</sup> nodule h <sup>-1</sup>	Ave	32.3	46.0	31.3	46.9	(9.01)

Residual of applied plant nutrients as mg kg<sup>-1</sup> soil were 0, 100 P, 200 K, and 200 tr with analysis of variance as General Linear Model, LSD .05 in parenthesis of each parameter.

growth was PKtr treatment, 1.40 g and the lowest yield 0.62 g was from tr treatment with LSD .05 of 0.21 g. The single application of P and combination with tr decreased leaf growth from 0.93 g for the control to 0.83 g, and 0.72 g, respectively. The leaf dry weight was enhanced when P was combined with K and Ktr as PK 1.01 g and PKtr 1.40 g. There was no effect to leaf growth when tr was associated with K. The yield was reduced when tr was applied alone and with P and resulting in 0.62 g for tr and 0.72 g for Ptr. The effect of applying K to the soil depressed leaf growth to 0.83 g with the control 0.93 g. The K combination with P and Ptr increased the yield and the yields were KP 1.01 g and PKtr 1.40 g. The yield was not affected by the combination of K and tr as 0.93 g.

The significantly highest stem dry weight, 1.87 g was obtained with the application of PKtr treatment with 0.19 g as LSD .05. The lowest stem dry weight was 0.40 g from tr treatment. P affected negatively to stem growth applied alone and combined with tr with 0.52 g for P and 0.46 g for Ptr and 0.53 g for the control. Stem growth was enhanced when P was applied in combination with K and Ktr as 1.02 g and 1.87 g respectively. The effect of applying K was positive in all treatments. For single addition K caused higher stem dry weight, 0.88 g with the control 0.53 g. When K was combined with P, tr, and Ptr yields were 1.02, 0.94, and 1.87 g, respectively. The addition of tr alone and with P decreased stem growth from 0.53 g of the control to 0.40 g of tr and 0.46 g of Ptr. When tr combined with K and PK the stem growth was Ktr 1.02 g and PKtr 1.87 g.

The significantly highest yield of root growth was 0.63 g with PKtr treatment, with the lowest of 0.26 g from tr treatment. The LSD .05 was 0.09 g. Applying P decreased root growth to 0.32 g from 0.42 g of the

check. Root growth also decreased when P was combined with trace elements with 0.33 g. However, P was added with K and tr resulted in 0.56, and 0.63 g, respectively. The effect of K when applied alone or in combination increased root development. With application of K only the yield was 0.53 g compared to 0.42 g for the control. K in association with P, tr, and Ptr increased root dry matter production, PK 0.56 g, Ktr 0.52 g, and PKtr 0.63 g. The single addition of tr to the soil decreased root dry weight with 0.26 g and the control 0.42 g. Also, root growth was depressed when tr was added with P as Ptr 0.33 g. However, the positive effect of combining tr with K and PK was noted as 0.52 g for trK and 0.63 g for PKtr.

Nodule fresh weight was significantly highest with PKtr of 1.19 g, with the lowest of 0.26 g of tr treatment using LSD .05 of 0.14 g. P alone increased nodule fresh weight to 0.49 g with the control of 0.47 g. P combined with tr yielded 0.31 g which was lower than the check, 0.47 g. However, when P was combined with K and Ktr increased fresh nodule weight to 0.63 g and 1.19 g, respectively. When the soil was treated with K alone or in combination, increased yields with K treatment was 0.55 g and KP, Ktr, and KPtr were 0.63, 0.58 and 1.19 g, respectively. Applying tr alone depressed fresh nodule weight with 0.29 g and the control of 0.47 g. Fresh nodule weight was decreased from 0.47 g of the control to 0.31 g due to trP treatment. Trace elements combined with K and PK increased yields as 0.58 g for Ktr and 1.19 g for PKtr.

The significantly highest nodule number was obtained with Ktr treatment, 56 nodules plant<sup>-1</sup> with the smallest of 9 from Ptr treatment with LSD .05 of 11.67 nodules plant<sup>-1</sup>. The addition of P with K and Ktr resulted in higher nodule number with PK 35 nodules plant<sup>-1</sup> and PKtr 49



nodules plant<sup>-1</sup> with the control 20 nodules plant<sup>-1</sup>. Applying P with tr decreased fresh nodule weight from 20 nodules plant<sup>-1</sup> for the control to 9 nodules plant<sup>-1</sup>. K effect was positive on nodule number when applied single or in combination with P, tr, and Ptr. With the control 20 nodules plant<sup>-1</sup>, K addition resulted in 47 nodules plant<sup>-1</sup>, PK 35 nodules plant<sup>-1</sup>, Ktr 56 nodules plant<sup>-1</sup>, and PKtr 49 nodules plant<sup>-1</sup>. Treating soil with trace elements depressed nodule number to 20 and 9 nodules plant<sup>-1</sup> when tr was applied alone and combined with P. However, number of nodule was increased from 20 for the control to 56 for Ktr and PKtr treatment.

The significantly highest nitrogenase activity was obtained with P treatment and the lowest was from tr treatment with LSD .05 of 9.01. P effect increased nitrogenase activity from 31 for the control to 49.6 C<sub>2</sub>H<sub>4</sub> u mole g<sup>-1</sup> nodule h<sup>-1</sup>. When comparing P effect combined with K, tr, and Ktr resulted in PK 42.3, Ptr 46.5, and PKtr 47.3 C<sub>2</sub>H<sub>4</sub> u mole g<sup>-1</sup> nodule h<sup>-1</sup>. The addition of K caused higher nitrogenase activity 42.3 with the control of 31. K effect when combined with P, tr, and Ptr was also positive resulting in 42.3, 40.6, and 47.3, respectively. Trace elements were depressive to nitrogenase activity as 22 when applied alone. Trace elements combined with K, P, and KP increased nitrogenase activity from 31 for the control to 46.5 for Ptr, 48.6 for Ktr, and 47.3 for PKtr.

Correlation coefficients of measured parameters as influenced by soil treatment of P, K, and tr factorial combination for Series I ( fertilized ) and Series II ( residual ) are summarized in Table XVIII.

Significant correlation coefficients for Series I were nitrogenase X leaf (0.561\*), nitrogenase X fresh nodule weight (0.469\*),

TABLE XVIII  
CORRELATION COEFFICIENTS OF PLANT GROWTH PARAMETERS,  
NITROGENASE, AND NODULATION OF WINGED BEAN AS  
AFFECTED BY P, K, AND tr COMBINATION

Series I						
Parameter	Stem	Leaf	Root	Nod No	Nod Wt	Nase
Stem		0.6805**	0.7234**	0.4372*	0.7511**	0.2681
Leaf	0.8656**		0.4656*	0.2290	0.6968**	0.5619*
Root	0.8316**	-0.3855		0.2989	0.5698*	0.0781
Nod No	0.7700**	-0.6544**	0.7265**		0.7215**	0.1033
Nod Wt	0.9214**	-0.4436*	0.7091**	0.7205**		0.4697*
Nase	0.1746	-0.2514	0.0335	0.0758	0.2915	
Series II						

\*, \*\* = Significant at P = 0.05 and 0.01, respectively  
Plant nutrients applied for Series I  
Residual response of applied nutrients for Series II

fresh nodule weight X stem (0.751\*\*), fresh nodule weight X leaf (0.696\*\*), fresh nodule weight X root (0.569\*), fresh nodule weight X number of nodule (0.721\*\*), nodule number X stem (0.437\*), root X stem (0.723\*\*), root X leaf (0.465\*), and stem X leaf (0.680\*\*).

Residual effects in Series II significant coefficients were apparent for fresh nodule weight X number of nodule (0.720\*\*), fresh nodule weight X root (0.709\*\*), fresh nodule weight X stem (0.921\*\*), and fresh nodule weight X leaf (-0.443\*). The correlation of number of nodule were with root, leaf and stem as 0.726\*, -0.654\*, and 0.770\*\*, respectively. The significant correlation coefficient between root and stem was 0.831\*\* while the correlation coefficient between stem and leaf was 0.865\*\*.

## CHAPTER V

### SUMMARY AND CONCLUSION

The principal objective of this study was to evaluate the effects of soil fertility treatments on growth, nodulation, nitrogenase activity, and nodule compositions of Winged Bean (Psophocarpus tetragonolobus) grown on dark red latosol soil from Brazil.

A second objective was to determine the influence of mycorrhiza with and without slowly soluble phosphorus on growth, nodulation, and nitrogenase activity levels.

Winged Bean (Psophocarpus tetragonolobus) has recently received attention as a high protein and high nitrogen-fixing crop. This plant grows throughout the neotropical countries, Asia, and Africa and can reduce protein deficiency in human diets. Also, all parts of winged bean are edible including seed, pod, leaf, flower, and tuber.

The dark red latosol soil (Typic Eutruxox, isohyperthermic, fine kaolinitic) used in this study has typical characteristics of tropical soils where winged bean is grown. This soil is acidic, low available phosphorus, low bases, low organic matter but high levels of Fe and Al saturation.

Five greenhouse experiments are reported in this study. The experiments were all conducted as completely randomized designs with 3 replications and 2 series. The second series was the residual effect of

the first series. Seeds were inoculated with 3 ml of a liquid medium containing viable cells of Rhizobium leguminosarum and germinated in each pot with one plant per pot.

The first experiment was conducted to study the effect of endomycorrhiza ( Glomus fasciculatum ) with and without sparingly soluble phosphorus on growth, nodulation, and nitrogenase activity levels. Low soluble  $\text{Ca}_3(\text{PO}_4)_2$  was applied as 0 and 100 ppm. Results indicated that endomycorrhiza had a significant influence on all parameters studied. Endomycorrhiza was beneficial for increased plant growth, nodulation, and nitrogenase levels of winged bean plant. However, endomycorrhiza in combination with slowly soluble phosphorus depressed root growth but increased stem and leaf growth. Therefore, phosphorus was beneficial to top growth but not for root growth. Nodule number was decreased but nodule fresh weight and nitrogenase activity levels were increased by endomycorrhiza with sparingly soluble phosphorus. It can be stated that added phosphorus was not essential for nodule setting but was necessary for increased nodule growth and nitrogenase activities. The effect of plant age was also studied with this experiment. Results obtained showed that fresh nodule weight increased with plant age. Despite the fact that total number of nodules  $\text{plant}^{-1}$  was not affected by plant age, the increase in the nodule weight was more directly related to nodule growth. Nevertheless, nitrogenase activity linearly decreased with plant age. Therefore, phosphorus did not increase nitrogenase activity by the way of increased nodule weight but was involved in physiological activity of nodules. Also, added phosphorus was necessary for increased top growth.

A second experiment was carried out to determine the effects of four P levels with and without K on growth, nodulation, nitrogenase

activity and nodule cytosol compositions. Fertility treatments consisted of 0, 100, 200, and 300 mg P kg<sup>-1</sup> soil and 0 and 200 mg K kg<sup>-1</sup> soil. Phosphorus levels mostly increased plant growth with and without K combination except for root growth within the residual effect series. However, K in combination with P levels generally produced higher plant dry matter than P levels alone. P levels with and without K did not increase number of nodules. Nodule weight and nitrogenase activity was increased with P levels alone and in combination with K. K combined with P levels caused higher nodule weight and nitrogenase activity levels. Therefore, high levels of P with K favored higher nodule weight and nitrogenase activity and P and K were essential for nodule growth not nodule setting. Nodule cytosol composition included P, K, Ca, Na, Mg content, aspartate transaminase, and ureide. P levels increased P, K, Na content and aspartate transaminase enzyme activity. K combined with P levels increased K, Ca content, and aspartate transaminase over P levels alone. Mg cytosol content and ureide were decreased by P levels with and without K combination. The conclusion can be drawn from these results that P addition was inhibitory with Ca and Mg and K was depressive with Na.

The third experiment was conducted to detect the effects of P, K, Ca and factorial combination on growth, nodulation, nitrogenase activity levels, and nodule cytosol compositions. The soil was treated with plant nutrients in factorial combination as mg kg<sup>-1</sup> soil of 0, 1200 Ca, 200 P, and 200 K. Phosphorus showed a negative effect on plant growth. K and Ca applied alone or in combination increased winged bean growth. Calcium increased number of nodules when applied alone or in combination with P and K but P and K did not affect number of nodules. Nodule weight and

nitrogenase activity increased when P, K, and Ca were applied alone and in combination. Therefore, Ca was necessary for nodule setting and all these three elements were needed for nodule growth and high nitrogenase enzyme activity. P, K, and Ca applied alone or in combination increased P, K, Na, Ca content, aspartate transaminase, and ureide but decreased Mg cytosol content. K combined with P depressed P cytosol content.

In the fourth experiment the effects of four K levels with and without P were studied on growth, nodulation, and nitrogenase activity. In this experiment plant nutrients included 0, 200, 400, and 600 mg K kg<sup>-1</sup> soil and 0 and 200 mg P kg<sup>-1</sup> soil. Plant growth was increased with K levels, whereas, P combined with K did not increase growth of this plant. Nodule number was depressed by K levels without P combination. K levels increased nodule weight and nitrogenase activity but nodule weight and nitrogenase activity were decreased when P was combined with K levels. It can be seen that high K levels were required for high nitrogen fixation.

The fifth experiment was to determine the effects of P, K and trace elements in factorial combination on growth, nodulation and nitrogenase activity of winged bean plants. Applied plant nutrients as mg kg<sup>-1</sup> soil were 0 and 100 P; 0 and 200 K; and 0 and 200 trace elements. Phosphorus was not beneficial to any plant growth parameter unless it was combined with K and/or trace elements. Single or combination application of K and trace elements increased winged bean growth and development. However, only combination application of these three elements increased plant growth due to the residual effects. Application of P, K, and trace elements single or combination increased nodule number but phosphorus showed the least effect on this parameter. In contrast with residual

effects only the combination of P, K, and trace elements increased total nodule number plant<sup>-1</sup>. Phosphorus showed a benefit on nodule weight and nitrogenase activity when combined with K and/or trace elements.

Residual effects were apparent on nodule weight and nitrogenase activity only combined P, K, and trace elements application. Therefore, P, K, and trace elements were all required for higher growth, nodulation, and nitrogenase activity levels.

Results from these studies have practical application toward increased winged bean production and symbiotic nitrogen fixation with improved soil fertility treatment. Phosphorus levels in combination with K are required for increased growth, nodulation, and nitrogenase activity. K levels favored high nitrogen fixation. Adequate available soil potassium, phosphorus, calcium, and trace elements are needed for increased growth, nodulation, and nitrogenase activity levels. Nodule cytosol composition, aspartate transaminase, and ureide content are indicative parameter for high symbiotic nitrogen fixation. Nodule cytosol ureides are of interest because of their role as a nitrogenous nonproteins component of accumulation and efficient translocation for winged bean plant.



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2  
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